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Effects of brood size on and the ontogeny of the stress response in nestling Tree Swallows (Tachycineta bicolor)

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Effects of brood size on and the ontogeny of the stress response in nestling Tree Swallows (*Tachycineta bicolor*)

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ABSTRACT

Altricial bird species rely on their parents to bring food, defend the nest and to help them thermoregulate. Brood size may influence how well adults are able to provision their young. A low quality diet has been shown to have long-term developmental costs and usually results in elevated corticosterone levels, which may mediate these costs. Chicks from larger broods may experience costs from poor condition and elevated corticosterone levels. My thesis addresses the questions of how natural brood size in Tree Swallows (*Tachycineta bicolor*) affects parental effort, chick growth and corticosterone levels, and at what age Tree Swallow chicks respond to a handling stress. Parents increase their effort, but not enough to match brood size, and chicks from larger broods are smaller in mass, but did not exhibit elevated CORT levels. Chicks respond with elevations in CORT at all ages sampled, but younger chicks have lower stress levels of CORT.

CHAPTER 1
INTRODUCTION

The effects of nutrition on growth and development have been of interest for several decades. A range of nutrition manipulation experiments have been conducted in rats, poultry, livestock, birds and even in humans and have found that poor early nutrition (i.e., during the developmental period) can have severe negative effects on the development of young (McCance and Widdowsen, 1962; Cravioto, et al., 1966; Negro, et al., 1994; Carsia and Mcilroy, 1998; Lucas, 1998; Lepczyk and Karasov, 2000). Growth rates are typically reduced and, in severe cases, the overall morphology between siblings in different nutrition experiments can be drastically different (McCance and Widdowsen, 1962). It has also been shown that poor nutrition can have profound effects on cognitive abilities. Children that were malnourished exhibited impaired cognitive abilities based on IQ tests (Cravioto et al., 1966). Gestating rats fed a low protein diet had young that were smaller at 2.5 months of age than young from rats fed *ad libitum* (control treatment) and this difference remained at 14 months of age. These smaller rats also made significantly more mistakes in a maze with a food reward than the control rats (Simonson and Chow, 1970). Rat pups that were raised in experimentally enlarged litters or fed a poor quality diet after weaning made significantly more errors in a Y-shaped water maze (Barnes, et al., 1966).

Compensatory growth is seen in many organisms following a restricted diet period (Metcalfe and Monaghan, 2001; Mangel and Munch, 2005; but see Fisher, et al., 2006). Originally thought to be an adaptive response once optimal conditions resume, compensatory growth may have associated long-term costs (Metcalfe and Monaghan, 2001). Poor nutrition during key developmental times is thought to result in investment in vital functions (e.g., brain development in humans and reproduction in insects) at the expense of other functions that may have less immediate influence on fitness but are nonetheless long-term costs (Metcalfe and Monaghan, 2001; Mangel and Munch, 2005). Compensatory growth can occur by accelerated growth rates or by taking more time to reach the same developmental stage, once adequate nutrition is acquired. What tissues are affected can vary drastically depending on the time during development that nutritional deficits occur (Mangel and Munch, 2005). Other

forms of compensation seen in amphibians and insects, for example, are an increase in the speed of progression through metamorphosis and pupation (Metcalfe and Monaghan, 2001; Mangel and Munch, 2005).

Periods of fasting or restricted food are associated with elevated baseline corticosterone levels (cortisol in humans and primates) (Wingfield and Kitaysky, 2002; Lynn, et al., 2003; Kitaysky, et al., 2006; Jenni-Eiermann, et al., 2008). The hypothalamic-pituitary-adrenal (HPA) axis regulates the release of glucocorticoids, including corticosterone. Corticosterone (CORT) is vital to life and without it an organism will not survive. This is due to CORT's role in metabolism and maintaining plasma glucose levels (Siegel, 1980). When glucose levels drop below homeostatic levels, CORT levels increase and facilitate mobilization of stored energy from the liver and muscle (Defeo, et al., 1989; Brillon, et al., 1995). This is a clear adaptive response to short-term fasts or when energy expenditure is high, such as during a predatory/prey interaction or short social conflict. CORT has other functions as well in humans: it helps modulate excitability and activity of neurons, helps maintain blood pressure (Bamberger, et al., 1996) and affects filtration in the kidneys (Samson, 1999).

There are also influences of CORT on a suite of vertebrate behaviors. Prolonged elevation of CORT levels during the breeding season will usually result in a shift from parental and reproductive behaviors to self-maintenance. Elevated CORT levels are associated with increased foraging and locomotor activity (Astheimer, et al., 1992; Lynn et al., 2003; Angelier, et al., 2007). CORT has also been shown to facilitate memory formation and retrieval (reviewed in Pravosudov, 2003). There are, however, costs to maintaining chronically elevated CORT levels.

A variety of costs have been reported in studies investigating the effects of prolonged elevations of CORT. Individuals with chronically elevated CORT levels are more susceptible to disease due to suppression of the immune system (reviewed in McEwen, et al., 1997; Sapolsky, 2000; Loiseau, et al., 2008). Individuals also experience high energetic demands due to the mobilization of fuels (Siegel, 1980; Sapolsky, 2000), and there appear to be effects

on developmental trajectories, cognitive abilities and overall reduced fitness (Kitaysky, et al., 2003; Fisher et al., 2006; Kitaysky et al., 2006; Hull, et al., 2007; Spencer and Verhulst, 2007; Loiseau et al., 2008; Wada and Breuner, 2008).

There may be a trade-off in developing young between responding to a stressor with an increase in CORT and the potentially negative effects of the elevations in CORT. Studies have demonstrated a hyporesponsive period to stress in rat pups (reviewed in Sapolsky and Meaney, 1986) and in rainbow trout (Barry, et al., 1995). During early stages of development young respond to a stressor with lower levels of glucocorticoids than do young later in development or as adults. It is thought that this hypo-responsive period is due to repression of the HPA axis and is adaptive to avoid the potential costs of elevated CORT levels during critical developmental stages (Barry et al., 1995; Sapolsky, 2000).

There have been few studies on birds investigating the development of the stress response, but those published have found chicks early in the nestling period have lower stress response CORT levels than older chicks (Schwabl, 1999; Sims and Holberton, 2000; Sockman and Schwabl, 2001; Love, et al., 2003; Fridinger, et al., 2007; Wada, et al., 2007; Quillfeldt, et al., 2009). It is unclear whether this is due to an undeveloped or repression of the HPA axis.

The objective of my thesis was to investigate the effects of natural brood size on growth trajectories and CORT levels and the ontogeny of the stress response in the chicks of a passerine species. Tree Swallows (*Tachycineta bicolor*) have variable brood sizes and have altricial young making them an ideal study species. Their chicks are completely dependent on the parents to bring food and defend the nest. Parents are aerial insectivores that may experience dramatic fluctuations in the food supply, which is likely a significant stressor to the chicks. Chicks may experience a trade-off between responding to a stressor to aid in surviving through the event and the potentially negative effects of chronically elevated CORT.

BIBLIOGRAPHY

Angelier, F., Clement-Chastel, C., Gabrielsen, G. W., and Chastel, O. (2007). Corticosterone and time-activity budget: an experiement with black-legged kittiwakes. *Hormones and Behavior* **52,** 482-491.

Astheimer, L. B., Buttemer, W. A., and Wingfield, J. C. (1992). Interactions of corticosterone with feeding, activity and metabolism in passerine birds. *Ornis Scandinavica* **23,** 355-365.

Bamberger, C. M., Schulte, H. M., and Chrousos, G. P. (1996). Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. *Endocrine Reviews* **17**(3)**,** 245-261.

Barnes, R. H., Cunnold, S. R., Zimmerma.Rr, Simmons, H., Macleod, R. B., and Krook, L. (1966). Influence of Nutritional Deprivations in Early Life on Learning Behavior of Rats as Measured by Performance in a Water Maze. *Journal of Nutrition* **89**(4)**,** 399-&.

Barry, T. P., Malison, J. A., Held, J. A., and Parrish, J. J. (1995). Ontogeny of the cortisol stress response in larval rainbow trout. *General and Comparative Endocrinology* **97,** 57-65.

Brillon, D. J., Zheng, B., Campbell, R. G., and Matthews, D. E. (1995). Effect of Cortisol on Energy-Expenditure and Amino-Acid-Metabolism in Humans. *American Journal of Physiology-Endocrinology and Metabolism* **31**(3)**,** E501-E513.

Carsia, R. V., and Mcilroy, P. J. (1998). Dietary protein restriction stress in the domestic turkey (Meleagris gallopavo) induces hypofunction and remodeling of adrenal steroidogenic tissue. *General and Comparative Endocrinology* **109**(1)**,** 140-153.

Cravioto, J., DeLicardie, E. R., and Birch, H. G. (1966). Nutrition, growth and neurointegrative development: an experimental and ecologic study. *Pediatrics* **38,** 319-320.

Defeo, P., Perriello, G., Torlone, E., Ventura, M. M., Fanelli, C., Santeusanio, F., Brunetti, P., Gerich, J. E., and Bolli, G. B. (1989). Contribution of Cortisol to Glucose Counterregulation in Humans. *American Journal of Physiology* **257**(1)**,** E35-E42.

Fisher, M. O., Nager, R. G., and Monaghan, P. (2006). Compensatory growth impairs adult cognitive performance. *Plos Biology* **4**(8)**,** 1462-1466.

Fridinger, R. W., O'Reilly, K. M., Kildaw, S. D., and Buck, C. L. (2007). Removal of a nest-mate elicits an age-dependent increase in plasma corticosterone of nestlng black-legged kittiwakes. *Journal of Field Ornithology* **78**(1)**,** 93-99.

Hull, K. L., Cockrem, J. F., Bridges, J. P., Candy, E. J., and Davidson, C. M. (2007). Effects of corticosterone treatment on growth, development, and the corticosterone response to handling in young Japanese quail. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* **148**(3)**,** 531-543.

Jenni-Eiermann, S., Glaus, E., Gruebler, M., Schwabl, H., and Jenni, L. (2008). Glucocorticoid response to food availability in breeding barn swallows (*Hirundo rustica*). *General and Comparative Endocrinology* **155,** 558-565.

Kitaysky, A. S., Kitaiskaia, E. V., Piatt, J. F., and Wingfield, J. C. (2003). Benefits and costs of increased levels of corticosterone in seabird chicks. *Hormones and Behavior* **43,** 140-149.

Kitaysky, A. S., Kitaiskaia, E. V., Piatt, J. F., and Wingfield, J. C. (2006). A mechanistic link between chick diet and decline in seabirds? *Proceedings of the Royal Society B-Biological Sciences* **273**(1585)**,** 445-450.

Lepczyk, C. A., and Karasov, W. H. (2000). Effect of ephemeral food restriction on growth of house sparrows. *the Auk* **117**(1)**,** 164-174.

Loiseau, C., Sorci, G., Dano, S., and Chastel, O. (2008). Effects of experimental increase of corticosterone levels on begging behavior, immunity and parental provisioning rate in house sparrows. *General and Comparative Endocrinology* **155**(1)**,** 101-108.

Love, O. P., Bird, D. M., and Shutt, L. J. (2003). Plasma corticosterone in american kestrel siblings: effects of age, hatching order, and hatching asynchrony. *Hormones and Behavior* **43,** 480-488.

Lucas, A. (1998). Programming by early nutrition: An experimental approach. *Journal of Nutrition* **128**(2)**,** 401S-406S.

Lynn, S. E., Breuner, C. W., and Wingfield, J. C. (2003). Short-term fasting affects locomotor activity, corticosterone, and corticosterone binding globulin in a migratory songbird. *Hormones and Behavior* **43**(1)**,** 150-157.

Mangel, M., and Munch, S. B. (2005). A life-history perspective on short- and long-term consequences of compensatory growth. *American Naturalist* **166**(6)**,** E155-E176.

McCance, R. A., and Widdowsen, E. M. (1962). Nutrition and growth. *Proceedings of the Royal Society of London: B* **156**(964)**,** 326-337.

McEwen, B. S., Biron, C. A., Brunson, K. W., Bulloch, K., Chambers, W. H., Dhabhar, F. S., Goldfarb, R. H., Kitson, R. P., Miller, A. H., Spencer, R. L., and Weiss, J. M. (1997). The role of adrenocorticoids as modulators of immune function in health and disease: Neural, endocrine and immune interactions. *Brain Research Reviews* **23**(1-2)**,** 79-133.

Metcalfe, N. B., and Monaghan, P. (2001). Compensation for a bad start: grow now, pay later? *Trends in Ecology & Evolution* **16**(5)**,** 254-260.

Negro, J. J., Chastin, A., and Bird, D. M. (1994). Effects of short-term food deprivation on growt of hand-reared american kestrels. *The Condor* **96**(3)**,** 749-760.

Pravosudov, V. V. (2003). Long-term moderate elevation of corticosterone facilitates avain food-caching behaviour and enhances spatial memory. *Proceedings of the Royal Society B-Biological Sciences* **270,** 2599-2604.

Quillfeldt, P., Poisbleau, M., Chastel, O., and Masello, J. F. (2009). Acute stress hyporesponsive period in nestling thin-billed prions *Pacyptila belcheri*. *Journal of Compartive Physiology: A* **195,** 91-98.

Samson, W. K. (1999). Adrenomedullin and the control of fluid and electrolyte homeostasis. *Annual Review of Physiology* **61,** 363-389.

Sapolsky, R. M. (2000). Stress hormones: Good and bad. *Neurobiology of Disease* **7**(5)**,** 540-542.

Sapolsky, R. M., and Meaney, M. J. (1986). Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. *Brain Research Reviews* **11,** 65-76.

Schwabl, H. (1999). Developmental changes and among-sibling variation of corticosterone levels in an altricial avian species. *General and Comparative Endocrinology* **116,** 403-408.

Siegel, H. S. (1980). Physiological Stress in Birds. *Bioscience* **30**(8)**,** 529-534.

Simonson, M., and Chow, B. F. (1970). Maze Studies on Progeny of Underfed Mother Rats. *Journal of Nutrition* **100**(6)**,** 685-&.

Sims, C. G., and Holberton, R. L. (2000). Development of the corticosterone stress response in young northern mockingbirds (*Mimus polyglottos*). *General and Comparative Endocrinology* **119,** 193-201.

Sockman, K. W., and Schwabl, H. (2001). Plasma corticosterone in nestling american kesterls: effects of age, handling stress, yolk androgens, and body condition. *General and Comparative Endocrinology* **122,** 205-212.

Spencer, K. a., and Verhulst, S. (2007). Delayed behavioral effects of postnatal exposure to corticosterone in the zebra finch (Taeniopygia guttata). *Hormones and Behavior* **51**(2)**,** 273-280.

Wada, H., and Breuner, C. W. (2008). Transient elevation of corticosterone alters begging behavior and growth of white-crowned sparrow nestlings. *Journal of Experimental Biology* **211**(10)**,** 1696-1703.

Wada, H., Hahn, T. P., and Breuner, C. W. (2007). Development of stress reactivity in white-crowned sparrow nestlings: total corticosterone response increases with age, while free corticosterone response remains low. *General and Comparative Endocrinology* **150,** 405-413.

Wingfield, J. C., and Kitaysky, A. S. (2002). Endocrine responsed to unpredictable envronmental events: stress or anti-stress hormones. *Integrative and Compartive Biology* **42,** 600-609.

CHAPTER 2
EFFECTS OF BROOD SIZE ON CHICK-FEEDING RATES, GROWTH AND CORTICOSTERONE IN NESTLING TREE SWALLOWS (TACHYCINETA BICOLOR)

INTRODUCTION

Altricial chicks depend on their parents to bring food and defend the nest. In species with large or variable clutch size the ability of the parents to care for the young may be affected by the number of chicks in the nest. There may be a greater division of resources between chicks if parents are not able to compensate their effort to match brood size.

The role that nutrition has during the nestling stage of birds has received increasing attention in the past few years due to the negative effects poor nutrition has on the developing young. Song is a sexual signal thought to reflect the quality of the singing male (Nowicki, et al., 1998). European Startling (*Sturnus vulgaris*) males that experienced unpredictable short-term food deprivation after independence spent significantly less time singing, had fewer song bouts and sang shorter songs in adulthood than males that were fed an *ad libitum* diet (Buchanan, et al., 2003). This supports the idea that song can reflect the past developmental stress of an individual (Buchanan et al., 2003). Nowicki et. al. (2000) also found evidence suggesting developmental condition in male Great Reed Warblers (*Acrocephalus arundinaceus*) has an influence on song development. Males in poorer developmental condition had a smaller song repertoire in adulthood. Blount et. al. (2003) showed that poor nutrition during the nestling period in Zebra Finches (*Taeniopygia guttata*) resulted in significantly lower plasma levels of lipophilic antioxidants (carotenoids, vitamins A and E) in adulthood. These antioxidants are an important defense against free radical damage, and carotenoids are important for male coloration in Zebra Finches, suggesting that the diet during the nestling period can permanently affect the ability of the birds to assimilate lipophilic antioxidants from their diet.

Another study on Zebra Finches examined the effect of nutrition during the nestling period on adult reproduction (Blount, et al., 2006). Zebra Finches reared on a low quality diet had

impaired reproductive capacity: they took longer to initiate a nest and laid eggs at a slower rate. Interestingly, clutch mass or size and yolk antioxidant levels were not affected, indicating the quality of the eggs was not affected. Because Zebra Finches are opportunistic breeders and timing is critical to reproductive success, these birds may have reduced fitness in their natural environment when favorable breeding conditions may be short-lived.

Studies conducted on American Kestrels (*Falco sparverius*) have looked at the effects of long-term restricted diets. Lacombe (1994) found that American Kestrel nestlings raised on restricted diets grew slower, but reached a similar asymptotic mass as the control nestlings raised on an *ad libitum* diet. The nestlings fed restricted diets had lower body mass at fledging, but fledged at the same age. Negro et. al. (1994) examined the effects of short-term food deprivation on American Kestrels. Chicks that were starved for 2-4 days experienced significant weight loss, but were able to make up the weight loss by eating more than controls when *ad libitum* food was restored. This particular study found no evidence of long-term effects on growth (body mass and lengths of the antebrachium, tarsus and beak).

There appears to be considerable variation in the effects nutrition has on patterns of growth, depending on the timing, duration and severity of the restricted diet period. In some cases, as mentioned previously, studies have reported that individuals reared on a poor diet are able to regain mass or catch-up to individuals reared on a high quality diet, once they are fed the high quality diet. A few studies have been conducted to determine if there are costs to this catch-up or compensatory growth.

A few studies have examined the effects of compensatory growth following poor quality nutrition during development on cognitive abilities in birds. These studies have generally concluded that poor nutrition results in reduced cognitive abilities (Metcalfe and Monaghan, 2001; Fisher, et al., 2006; Kitaysky, et al., 2006). Kitaysky et. al. (2006) found that Red-legged Kittiwake (*Rissa brevirostris*) chicks fed a low quality diet performed significantly worse in an associative learning task (associating the color of food dishes with the presence or absence of food) than chicks raised on a high quality diet. The chicks fed the low quality

diet also performed worse on a spatial cognitive task (escape from an enclosure) after fledging than the chicks raised on the high quality diet. Fisher et. al. (2006) found that the associative learning abilities of Zebra Finches were negatively related to the amount of compensatory growth they experienced.

Diet has a strong influence on corticosterone levels, which has important roles in metabolism and maintaining plasma glucose levels. Corticosterone (CORT) also has strong influences on a suite of bird behaviors. During the breeding season many species of birds will attenuate their CORT levels to avoid nest abandonment (Wingfield, et al., 1995; Wingfield and Sapolsky, 2003; Love, et al., 2004). Birds that experience prolonged elevations in CORT during the breeding season will usually shift from parental and reproductive behaviors to self-maintenance. Elevated CORT levels are associated with increased foraging and locomotor activity (Astheimer, et al., 1992; Lynn, et al., 2003; Angelier, et al., 2007). In chicks, elevated CORT levels have been associated with increased begging (Kitaysky, et al., 2001; Kitaysky, et al., 2003) and Kitaysky et. al. (2003) found increased aggressive behavior in Black-legged Kittiwake chicks with experimentally elevated CORT levels. Elevated CORT levels have also been shown to facilitate memory formation and memory retrieval in birds (reviewed in Pravosudov, 2003). There are, however, costs to maintaining chronically elevated CORT levels.

Black-legged Kittiwake chicks with CORT levels elevated with subcutaneous implants during the nestling period had reduced cognitive abilities compared to chicks with blank implants (Kitaysky et al., 2003). Chicks with CORT implants increased their food intake, likely due to the high energy demands from the elevated CORT levels, but their masses were not different from control chicks. Male Zebra Finches that experienced prolonged elevations in CORT levels during the nestling period also experienced impaired song learning (Spencer, et al., 2003). These males then sang significantly shorter songs than control males, suggesting that the experimental males were of lower quality. These results are similar to results found in diet manipulation experiments, suggesting chronically elevated CORT levels could be the main cause of these observed negative effects.

Animals that have indeterminate clutch sizes may adjust the number of eggs based on their own condition during the period prior to laying and the current environmental conditions (Robins, 1970; Blancher and Robertson, 1987; Monaghan, et al., 1995). This plasticity in clutch and brood sizes is thought to help maximize the lifetime reproductive output of the adult birds by allowing them to invest optimally in reproduction so that they can successfully fledge young without jeopardizing survival (reviewed in Klomp, 1970). Female birds in poorer condition tend to lay fewer eggs or eggs in poorer condition (i.e., smaller in mass or having lower yolk antioxidant) and therefore have smaller broods, while female birds in good condition tend to lay more eggs and have larger broods (Houston, et al., 1983; Erikstad, et al., 1993). Lack (1947) hypothesized that clutch size evolved to correspond with the maximum number of young that parents can feed. More often, however, the most common brood size is smaller than the most productive brood size, indicating that there are other limitations or factors besides the number of chicks to feed that affect the resulting brood size (DeSteven, 1980; Boyce and Perrins, 1987). A review by Monaghan and Nager (1997) discusses studies that have investigated the limitations to clutch size. They review studies that have shown costs to producing larger clutches in several species: domestic chickens (*Gallus domesticus*), Barn Swallows (*Hirundo rustica*) and American Kestrels, but other studies that vary in their results to brood manipulations. Some studies report no effect to the adults or chicks of enlarged broods while a few report negative effects to the condition of the adults. Parents that were given more eggs to incubate early in the incubation period (e.g., Northern Gannets (*Morus bassanus*), Collared Flycatchers (*Ficedula albicollis*) and Common Terns (*Sterna hirundo*)) fledged fewer young than the non-manipulated clutches (Monaghan and Nager, 1997). DeSteven (1980) suggested that variation in parental ability in terms of foraging and general care of the young will lead to variation in clutch sizes. Adults that initiate a larger clutch do so because they are able to care for them, and therefore the chicks from larger clutches may not suffer because the parents are of better quality. Many studies have looked at the effect of brood size manipulations on the growth of the chicks and the fitness of the adults and, indeed, they have found that parents with experimentally enlarged broods suffer more in fitness (i. e., greater mass loss, increased CORT, lower survival) than adults from

control broods or experimentally reduced broods (DeSteven, 1980; Dijkstra, et al., 1990; Ilmonen, et al., 2003). Likewise, the chicks from experimentally enlarged broods tend to suffer in mass and growth rates than chicks from experimentally reduced and/or control broods and have greater mortality (DeKogel, 1997), indicating that the original brood sizes of these adults may have been optimal for overall offspring condition.

Very little has been published on the effect natural variation in brood size may have on variation in chick growth. A study by Spear and Nur (1994) examined the effects of brood size, hatch date, and hatch order on Western Gull (*Larus occidentalis*) nestling survival. They found that chicks from smaller broods survived better from hatch to fledging than chicks from larger broods, indicating there may be costs to larger brood sizes. Interestingly, a study by Loonen et. al. (1999) found a positive effect of brood size on mass and fledging in both natural and experimentally enlarged brood sizes in Barnacle Geese (*Branta leucopsis*). Families with larger brood sizes also occupied larger feeding areas and the families defended those areas better. It is important to keep in mind that these are a precocial species where the chicks are not bound to a nest, unlike altricial species where chicks are dependent on the parents to provide food.

Even though adults in better condition often lay larger clutches, adults cannot predict environmental perturbations that could affect food supply and/or foraging abilities throughout the nestling period. During periods of inclement weather or low food availability chicks in larger broods may suffer more than chicks in smaller broods. Parents raising larger broods may not be able to compensate during periods of poor food availability compared to parents with small broods, leading to the question of whether there are costs to the chicks raised in larger brood sizes. Chicks from larger broods may have reduced growth, be in poorer condition or be smaller in size or have long-term reduced cognitive abilities or other behavioral effects compared to chicks from smaller broods. Conversely, if parents are able to compensate by increasing chick-provisioning rate the chicks may not differ from those in small broods.

The objective of this study was to investigate potential costs to being raised in larger broods in terms of chick growth rate, mass, condition and CORT levels. Tree Swallows are an ideal species in which to study these effects because natural clutch sizes can vary from 2 to 7 eggs in a central Iowa population, they are secondary cavity nesters that nest readily in provided nest boxes and are aerial insectivores (Robertson, et al., 1992). Insect populations can fluctuate dramatically, potentially leading to periods of low prey abundance (Cucco and Malacarne, 1996) and foraging abilities can be affected by weather conditions (McCarty and Winkler, 1999). If there is an increase in cost with brood size, this leads to the following predictions: (1) nest visitation rates, which I assume is proportional to chick feeding rate, may increase with increasing brood size, but the per capita chick feeding rates will decrease; (2) chicks from larger broods will have smaller mass at a given age, be in poorer condition and have lower growth rates compared to chicks from smaller broods; and (3) chicks from larger broods will have elevated baseline CORT levels compared to chicks from smaller broods.

METHODS

Study Species

The Tree Swallow (*Tachycineta bicolor*) is a migratory passerine that breeds in the northern United States and Canada, typically from May to July (Robertson et al., 1992). It is a secondary-cavity nester that nests readily in nest boxes. Tree Swallows are aerial insectivores that prefer to nest in open areas near water. The central Iowa population has a clutch size range of 1-7 eggs with an average ± SE of 5.3 ± 1.0 eggs per clutch. Brood sizes have been documented to range between 1 to 6 chicks in this population with an average ± SE of 3.9 ± 2.1 chicks per brood, based on chicks that made it to fledging (Parsons, *pers. obs.*). Raising and fledging more than one brood in a season is rare, however re-nesting does occur if nest failure occurs early in the season. Only female Tree Swallows incubate and generally begin incubation on the day the penultimate egg is laid. Incubation lasts, on average, 14 days. Most clutches hatch over a 1-2 day period. Chicks fledge from the nest by 18-20 days post-hatch.

Study Site

The study was conducted using a wild population of Tree Swallows at three locations near Ames, Iowa with 205 nest boxes distributed between the sites. Each site contains access to prairie and water. The sites are located at Ada Hayden Park (42°32'N, 93°37'W), the ISU Horticulture Farm (42°6'N, 93°35'W) and Hinds Research Farm (42°3'N, 93°37'W). Nests were monitored every three days beginning in early May to record nest building activity. Once egg-laying began I checked the nests every 2-3 days to record the date of egg-laying and clutch size and then every day around the time of hatching (approximately 14 days after the start of incubation) to record the hatch date.

Measurements

I monitored 95 chicks in 22 nests in 2007 and 121 chicks in 29 nests in 2008. The measurements (see below) from individual nestlings within a nest were averaged for each nest to take into account the non-independence of nest-mates.

Nest Visitation Rates:

I estimated the rate at which chicks were fed by their parents (nest visitation rates) by counting the number of visits adults made to the nest box during a 30-minute period following a 10-20 minute acclimation period. Observations were made with the aid of binoculars while sitting at a minimum distance of ~20 meters from the nest box. Observations periods were distributed throughout the morning and early afternoon and time of day was included in the analyses; I did not make nest visitation rate observations during rain, when adults make very few visits to the nest. In 2007, I made observations on days 7 and 12 post-hatch at each nest. I chose days 7 and 12 post-hatch because of the time constraints and the lack of available help that year. In 2008, I made observations on days 7, 12 and 15 post-hatch. In 2007, males and females were identified and their visits to the nest recorded separately. Females were either identified by brown plumage (first year females) or by marking the females during the incubation period with white color on their rump and tail and black marks on their throat. Male and female Tree Swallows participate equally in nest visits ($F = 1.63$, p-value = 0.21) (Parsons, *pers. obs.*); therefore I did not mark adult birds in 2008 and lumped male and female feedings when analyzing visitation rates. Nest visitation

rates were ln-transformed before analyses because nest visitation rates were not normally distributed.

Morphological Measurements:
2007: I weighed chicks, using a 10 or 30 gram Pesola scale readable to 0.1g and 0.25g, respectively, on days 3, 7, 12 and 15 post-hatch. I also measured head-bill length on day 12 post-hatch. Head-bill was measured as the maximum distance between the cerebellar prominence on the back of the head and the tip of the bill, using calipers readable to 0.1 mm. The chicks were also banded with a metal USGS leg band on day 12. Prior to banding, I identified individual chicks by unique nail polish colors on all claws of one foot.

2008: The same protocol was followed with the addition of measuring the head-bill length on days 3, 7, 12 and 15, at the same time the chicks were weighed.

As an indicator of body condition I used size-corrected body mass. I used head-bill length as a measure of size and used the residuals from the regression of mass versus head-bill measured across all chicks of the same age as our measure of condition (Fig. 2.1). I calculated a separate regression for each measurement day. A Gompertz logistic growth curve was fitted to the mass measurements from days 3, 7, 12 and 15 post-hatch of all chicks in each nest (Fig 2.2) (McCarty, 2001). The equation for this curve allowed us to calculate the mean growth rates and the predicted asymptotic mass for each nest, as well as the time to reach the predicted asymptotic mass.

Blood Samples:
Blood samples (~50 ul) were taken from the chicks for the analysis of plasma corticosterone (CORT) levels. Blood was taken from the brachial vein and collected in heparinized microhemotocrit tubes, which were kept on ice until I returned to the lab within 7 hours. In the lab, I spun the blood in a hematocrit centrifuge and transferred the plasma to 0.5 ml centrifuge tubes, which were stored in at -80^{o}C until analyzed. I took blood samples from

each chick within a nest on day 12. Samples for baseline CORT levels were collected within 3 minutes of first opening the nest box and disturbing the chicks.

Weather Parameters:

Weather data for Ames, IA, located at 42° 1'N, 93° 47'W, were obtained from the Iowa Ag Climate Network (http://mesonet.agron.iastate.edu/agclimate/index.phtml). I acquired hourly and daily weather data from the summers of 2007 and 2008, which included temperature (average high and low temperature for the daily weather data), precipitation, wind speed and relative humidity.

Corticosterone Assay

I assayed plasma concentrations of CORT using a double antibody I^{125} radioimmunoassay (RIA) kit from MP Biomedicals (Catalog # 07-120103, MP Biomedical, Orangeburg, NY). Samples were run in triplicate each year. This kit has been validated for measuring plasma CORT concentrations in birds (Washburn, et al., 2002). I followed the protocol of the kit with the exception of quartering the volume of all the reagents, diluting the 25 ng/ml standard to 1:2 and 1:4 with steroid diluent to produce a 12.5 ng/ml standard and a 6.25 ng/ml standard. I also used a 1:40 dilution of plasma, using the provided steroid diluent in the assay. This dilution resulted in percent binding that fell consistently within the usable range provided by the standard curve. A pool of Tree Swallow plasma was used to test for binding parallelism with the standard curve (Fig. 2.3). The intra-assay variation was 6% and the inter-assay variation was 15%. The mean inter-assay variation was calculated using the average coefficient of variation of the control samples provided in the kit, while the intra-assay variation was calculated using the average coefficient of variation of each set of triplicates. The control samples were run with every set of samples through the assay. The plasma from each year was run in one assay at the end of each respective field seasons. The baseline CORT values were not normally distributed and were ln-transformed before analyses.

Statistical Analyses

All analyses were performed using JMP statistical software (SAS Institute 2007). I used the generalized linear model platform that uses a likelihood ratio chi-square (G^2) for all analyses. Model designs were based on including independent variables of interest (i.e., brood size) and other factors that are known to have effects on chick growth and adult foraging abilities (Dawson, et al., 2005; Dawson, 2008). Nests that did not hatch all eggs layed were included in the analyses. I did this due to the low sample sizes that would have resulted from excluding these nests. Several of the nests had eggs that did not hatch. For the purpose of this study I were primarily interested in the effects of brood size and not the effects of weather variables; therefore, I used principal component analyses to combine our weather data. The principal component analyses (PCA) of the weather data take into account weather variation during the season. The time interval over which weather data were included in the PCA's performed was dependent on the measurement in question. Two separate PCA's were calculated with the weather variables for the following analyses: 1) Hourly weather: I used hourly weather data during the range of dates, 14 June – 25 July, and times, 0630 – 1400 hours, when nest visitation rates were observed and blood samples were collected for the PCA. The principal components from the hour of the nest visitation measurement were used in the nest visitation analyses and the principal components from the hour prior to the collection of the blood sample were used in the analysis of baseline CORT and 2) Condition, growth rates and asymptotic mass: I used accumulated mean daily weather data during the range of dates, 2 June – 25 July, when the morphological measurements were taken. The mean of the principal components from day 0 (day of hatch) to the day of the measurement (e.g., to day 12) was used in the analyses. The principal component loadings of the two weather PCA's can be found in Table 2.1. A list of models used is provided in Table 2.2. Models were analyzed separately. Least square means used to construct the figures were calculated for brood size using a standard least squares analysis platform in JMP.

Results

Measurement values reported are the average values ± SD.

Nest Visitation Rates

Tree Swallows visited their nests approximately every 2-3 minutes during the 30-minute observation period (Table 2.3). Nest visitation rates were not strongly influenced by brood size early in the nestling season, but the effect was stronger as the nestling period proceeded (Table 2.4). Brood size had a significant positive effect on nest visitation rates on days 12 and 15 post-hatch. There were significant increases in nest visitation rates when comparing the 2-chick and 3-chick broods on days 12 post-hatch (Fig. 2.3a) and on days 15 post-hatch (Fig. 2.3b). On day 15 post-hatch, measured only in 2008, there were significant effects of Julian date, time of day and both weather principal components (Table 2.4).

Per Capita Chick Feeding Rates

Brood size affected the average number of times an individual chick was fed by the parents on days 7 and 15 post-hatch (Table 2.4). On day 7 there was a significant decrease in the per capita chick feeding rates when comparing the 2-chick and 3-chick broods (Fig. 2.4a). On day 15 post-hatch the per capita chick feeding rates for the 2-chick brood was significantly lower than for either the 1-chick or 3-chick broods, but this is likely due to low sample of the one 2-chick brood, given that this nest had very low feeding rates (Fig. 2.4c).

On day 15 post-hatch the per capita chick feeding rates were also affected by Julian date, time of day and both weather principal components (Table 2.4). The model for day 12 per capita chick feeding rates was non-significant with no effect of brood size.

Mass

Chicks from all brood sizes were of similar mass early in nestling period (Fig. 2.5a, Table 2.5), but as the nestling period proceeded, chicks from larger broods were smaller on any given day (Fig. 2.5b, c, d, Table 2.5). Chicks grew quickly early in the nestling period nearly tripling in mass between day 3 and day 7 post-hatch. Chick growth slowed between day 7 and day 12 post-hatch, increasing 37% in mass over this interval. Chicks lost about 4% of their body mass between day 12 and day 15 post-hatch (Table 2.6). I observed a 6% difference in mass between chicks from the 4- and 5-chick broods on day 12 post-hatch and a 17% difference in chick mass between the 4- and 5-chick broods day 15 post-hatch.

The 1-chick broods were significantly larger than the 2-chick broods on day 7 post-hatch (Fig. 2.5b). There was a significant decrease in mass when comparing the 4-chick and 5-chick broods on days 7, 12, and 15 post-hatch (Fig. 2.5b, c, d). There was a significant effect of weather PC1 on the mass of the chicks on day 12 post-hatch (Table 2.5). On day 15 post-hatch there were also significant effects of weather PC1 and PC2 on chick mass.

Condition

In general, chicks from larger broods were in poorer condition than chicks from smaller broods (Table 2.7, Fig 2.6). Body condition on day 3 post-hatch (2008 only) did not vary with brood size, indicating that all chicks started at about the same condition, but there were significant negative effects of brood size on chick condition on days 7, 12 and 15 post-hatch. On day 7 post-hatch, there was a significant decrease in chick condition when comparing the 2-chick and 3-chick broods with a marginally significant increase in condition between the 3-chick to 4-chick broods (Fig. 2.6b). There was a marginal effect of Julian date on day 7 condition. On day 12 post-hatch, there was a significant decrease in condition when comparing the 4-chick and 5-chick broods (Fig. 2.6c). Year and the weather principal components also had significant effects on condition on day 12 post-hatch. On day 15 post-hatch condition decreased when comparing the 3-chick and 4-chick broods (Fig. 2.6d). There was also a significant effect of Julian date. I dropped time of day from the model on day 15 post-hatch because time was not well documented for all measurements.

Growth Rates and Asymptotic Mass

There was no effect of brood size on the calculated growth rates of the chicks (Table 2.8, Fig. 2.7), but there was a significant effect of brood size on the predicted asymptotic mass of the chicks. There was a significant effect of weather PC1 on the predicted asymptotic mass. The average growth rate and predicted average asymptotic mass were 0.39 ± 0.12 and $22.67\text{g} \pm 1.58$, respectively. There was no effect of brood size on the predicted age at which to reach asymptotic mass (g) was reached (Fig. 2.7c). The Gompertz logistic growth curve tended to over-estimate the predicted age to reach asymptotic mass (g).

Corticosterone

There was no detectable effect of brood size on the baseline CORT levels measured on day 12 (Table 2.9, Fig. 2.8). There was a significant year effect on baseline CORT with lower baseline CORT levels measured in 2007 than in 2008. The average baseline CORT levels of the chicks across the two years were 9.02 ± 6.25 ng/ml.

DISCUSSION

The natural variation in brood size that occurs in Tree Swallows has implications for chick growth and parental effort. Nest visitation rates of parents increased with brood size, but this increase was not proportional to the brood size, and the per capita chick feeding rate for chicks from the larger broods was reduced compared to chicks in small broods. This pattern of feeding effort has been seen in other altricial species for both manipulated and natural brood sizes (Martin, 1987). Parental feeding effort generally increased with brood size, but the per capita chick feeding rate decreased, indicating that birds modulate their parental effort, but not enough to compensate for large brood sizes (Martin, 1987). Dijkstra et. al. (1990) found a similar pattern in American Kestrels that experienced brood manipulations. Male parents of enlarged brood spent significantly more time hunting and brought more food to the enlarged broods, but the chicks from enlarged broods consumed significantly less food than the control and reduced broods. I found that weather PC1 and Julian date had significant effects on the chick provisioning rates, but since they are not consistent across the days it is difficult to interpret what these effects mean.

In our data there appears to be a threshold effect in feeding effort in Tree Swallows adults between the 2- and 3-chick broods for both the nest visitation rates and the estimated feeding rates per chicks, suggesting there may be a maximum feeding effort that Tree Swallows can expand in feeding their chicks. Interestingly, Murphy et. al. (2000) found a similar pattern in a brood manipulation experiment with Tree Swallows, but the threshold in their study occurred between the 6- and 7-chick broods. Murphy et. al. (2000) suggested that the year they collected the chick provisioning data was a high-resource year based on nest success. If

insect availability affects the maximum provisioning rate Tree Swallows can show, then this would suggest that there would be the temporal and spatial variability in parental responses to brood size. It appears that Tree Swallows adults are able to modulate their parental effort in response to brood size, but not enough to achieve equal per capita feeding rates across all brood sizes. Thus, chicks from large broods experience less favorable conditions than chicks from small broods, although the trade-off in thermoregulation may be another parameter to keep in mind. Dunn (1979) demonstrated that Tree Swallow chicks from smaller broods took longer to achieve thermoregulatory abilities than chicks from larger broods, possibly due to the larger broods having more chicks to keep each other warm. She found greater variation in the age the chicks were able to thermoregulate compared to a study conducted with House Wrens (*Troglodytes aedon*), which she attributed to the variability in the thickness of the feather layer found in Tree Swallow nests. Tree Swallows build their nests using grasses and line them with feathers. The number of feathers lining a nest can vary in a population and has been shown to have significant effects on the growth of the nestlings (Winkler, 1993). There may be costs to chicks from smaller broods in thermoregulation, particularly during cold periods, although I did not measure these costs or the feathers lining the nest.

Early in the nestling period chicks of all but the smallest brood sizes were of similar mass and condition. By day 7 post-hatch a negative correlation between either mass or body condition and brood size was apparent. This has also been reported in other studies of altricial species with either brood manipulation (DeSteven, 1980; Murphy et al., 2000; Shutler, et al., 2006; Saino, et al., 2008) or natural brood sizes (Perrins, 1965; Crossner, 1977; Bryant, 1978; Nur, 1984). The largest broods (5- and 6-chick) from our study appear to suffer the most in mass and condition as compared to chicks from smaller broods. I infer that the lower per capita feeding rate for chicks from larger broods resulted in their smaller mass, a difference that persisted until at least 15 days post-hatch. I could not measure the chicks after this time without risking premature fledging, so I cannot be certain if these differences in mass remained until fledging. It has been well documented in other passerines, however, that nestling mass and mass at fledging are positively correlated with post-fledging survival (Perrins, 1965; Garnett, 1981; Nur, 1984; Tinbergen and Boerlijst, 1990). Nur (1984)

conducted a brood manipulation in Blue Tits (*Parus caeruleus*) and found that post-fledging survival rates were lower for the largest broods compared to smaller broods. DeKogel (1997) also carried out a broods manipulation study in captive Zebra Finches and found that post-fledging mortality was highest in birds from enlarged broods. I observed a 17% difference in mass on day 15 post-hatch between the 4- and 5-chick broods, and although I found no effect of brood size on fledging success it is possible Tree Swallow chicks from larger broods suffer greater post-fledging mortality if they fledge at a reduced mass compared to chicks from small broods.

I expressed body condition as body mass normalized by head-bill length for chicks of the same age. Because growth of body components can vary (McCarty, 2001), however, and nutritional state can affect differential allocation to these components (Miller, *in prep*), it is not clear how best to estimate body condition for growing organisms. In our Tree Swallow data patterns in chick body condition were not as clear as the mass data. Nonetheless, there was a trend for condition to be negatively correlated with brood size. A brood manipulation experiment in Great Tits (*Parus major*) showed that the condition of the chicks (calculated as the residual from body mass regressed on tarsus length) also decreased with brood size (Neuenschwander, et al., 2003). On days 12 and 15 post-hatch there are, again, inconsistent patterns in the other variables that have significant effects on condition. There is variation in the condition data not explained by brood size. It is difficult to interpret the meaning of these variables.

Contrary to what others have reported (Dijkstra et al., 1990), I found no effect of brood size on the calculated growth rates of chicks. I did, however, find that the predicted asymptotic mass was significantly negatively correlated with brood size. This suggests that the chicks from larger broods are reaching their asymptotic mass sooner than chicks from smaller broods, however, I were unable to find an effect of brood size on the predicted age to reach the asymptotic mass. This may be due to small sample sizes and low power for both the growth rate and the predicted age to reach the asymptotic mass; power was about 13% for these models. For our model of growth rate to have 80% power I would need a sample size of

about 584 broods to detect significance, at α-level = 0.05. The sample size needed is large and, for our population, unreasonable. The lower asymptotic mass in large broods is consistent with the mass data indicating that chicks from larger broods are lighter than chicks from small broods.

The relationship between brood size and baseline CORT levels in chicks is not well established, especially in natural broods. I would expect that since the chicks from larger broods were lighter, their CORT levels would be elevated relative to the chicks from the smaller broods. Despite the smaller mass and reduced body condition in chicks from larger broods, I found no relationship between brood size and baseline CORT levels. This may indicate that the reduced provisioning rate and lower body mass of chicks from large broods were not great enough to be recognized as a physiological stressor. It is not the case that the chicks measured in this study cannot respond with by increased CORT levels as Tree Swallow chicks from this same population were used in another study to investigate the ontogeny of the stress response, and I found that by 12 days post-hatch the chicks appeared to have a fully responsive stress response (Parsons, Chapter 2). Ilmonen et. al.(2003) found in a brood manipulation experiment in Pied Flycatchers (*Fidicula hypoleua*) that the chicks from control and enlarged broods on day 12 post-hatch tended to have elevated CORT levels relative to reduced broods. The fact that Tree Swallow chicks did not show elevated CORT levels may be due to their not being physiologically stressed. The 6% difference in mass between the 4- and 5-chick broods may not have been enough of a difference to be reflected in elevated baseline CORT levels. An alternative explanation may be that even though the total CORT levels I measured in our assay were not different, the free CORT may vary with nutritional state. 80-90% of the circulating CORT in the blood is bound by corticosterone-binding globulin (CBG) (Siegel, 1980; Wada and Breuner, 2008), which prolongs the half-life of the steroid but decreases the proportion of free hormone that can enter cells and bind to receptors (Siegel, 1980). Dempsey (2006) found that Red-legged Kittiwakes that were protein restricted had lower CBG levels, thereby increasing their free CORT (unbound by CBG) levels. Tree Swallow chicks may have varying CBG levels across the broods sizes, so that even though their total CORT levels do not vary, their free CORT levels may. Interestingly, I found no effect of time on baseline CORT levels. CORT levels exhibit a circadian rhythm

throughout the day and night (Weitzman, et al., 1971; Breuner, et al., 1999), so I would expect there to be an influence of time on the CORT levels. Given that I did not find an effect of time could indicate that these chicks have not developed a circadian rhythm of CORT secretion or I happened to obtain blood samples during a range of times that did not exhibit a circadian rhythm.

Brood size has negative effects on chick mass and condition, likely due to the lower per capita chick feeding rates in larger broods, but these effects were not strong enough to detect an effect on baseline CORT levels in our study. Others have suggested there are no costs to raising larger broods even though they find lower mass and even elevated CORT levels in chicks from larger broods (reviewed in Murphy et al., 2000). This may be true when considering the fitness costs of the parent who can raise more young with larger brood size. I argue that due to the smaller mass and lower condition of chicks from larger broods there are costs to the offspring of being raised in a larger brood. Even though increased brood sizes and decreased per capita chick feeding rates do not always translate into a decrease in fledging survival (Nur, 1984; Dijkstra et al., 1990), short-term survival does not necessarily equate to high fitness. A smaller than average bird may survive after fledging, but their future reproduction may suffer due to its developmental environment.

BIBLIOGRAPHY

Angelier, F., Clement-Chastel, C., Gabrielsen, G. W., and Chastel, O. (2007). Corticosterone and time-activity budget: an experiement with black-legged kittiwakes. *Hormones and Behavior* **52,** 482-491.

Astheimer, L. B., Buttemer, W. A., and Wingfield, J. C. (1992). Interactions of corticosterone with feeding, activity and metabolism in passerine birds. *Ornis Scandinavica* **23,** 355-365.

Blancher, P. J., and Robertson, R. J. (1987). Effect of Food-Supply on the Breeding Biology of Western Kingbirds. *Ecology* **68**(3)**,** 723-732.

Blount, J. D., Metcalfe, N. B., Arnold, K. E., Surai, P. F., Devevey, G. L., and Monaghan, P. (2003). Neonatal nutrition, adult antioxidant defences and sexual attractiveness in the zebra finch. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**(1525)**,** 1691-1696.

Blount, J. D., Metcalfe, N. B., Arnold, K. E., Surai, P. F., and Monaghan, P. (2006). Effects of neonatal nutrition an adult reproduction in a passerine bird. *Ibis* **148,** 509-514.

Boyce, M. S., and Perrins, C. M. (1987). Optimizing great tit clutch size in a fluctuating environment. *Ecology* **68**(1)**,** 142-153.

Breuner, C., Wingfield, J. C., and Romero, L. M. (1999). Diel rhythms of basal and stress-induced corticosterone in a wild, seasonal vertebrate, Gambel's white-crowned sparrow. *Journal of Experimental Zoology Part A: Comparative Experimental Biology* **284**(3)**,** 334-342.

Bryant, D. M. (1978). Establishment of weight hierarchies in the broods of house martin *delichon urbica*. *Ibis* **120,** 16-26.

Buchanan, K. L., Spencer, K. A., Goldsmith, A. R., and Catchpole, C. K. (2003). Song as an honest signal of past developmental stress in the European starling (Sturnus vulgaris). *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**(1520)**,** 1149-1156.

Crossner, K. A. (1977). Natural selection and clutch size in the european starling. *Ecology* **58,** 885-892.

Cucco, M., and Malacarne, G. (1996). Reproduction of the pallid swift (*Apus pallidus*) in relation to weather and aerial insect abundance. *Italian Journal of Zoology* **63,** 247-253.

Dawson, R. D. (2008). Timing of breeding and environmental factors as determinants of reproductive performance of tree swallows. *Canadian Journal of Zoology* **86,** 843-850.

Dawson, R. D., Lawrie, C. C., and O'Brien, E. L. (2005). The importance of microclimate variation in determining size, growth and survival of avian offspring: experimental evidence from a cavity nesting passerine. *Oecologia* **144,** 499-507.

DeKogel, C. H. (1997). Long-term effects of brood size manipulation on morphological development and sex-specific mortality of offspring. *Journal of Animal Ecology* **66**(2)**,** 167-178.

Dempsey, T. D. (2006). Stress physiolgoy and reproductive performance of red-legged kittiwakes (*Rissa brevirostra*), University of Alaska Fairbanks.

DeSteven, D. (1980). Clutch size, breeding success, and parental survival in the tree swallow (Iridoprocne-bicolor). *Evolution* **34**(2)**,** 278-291.

Dijkstra, C., Bult, A., Bijlsma, S., Daan, S., Meijer, T., and Ziflstra, M. (1990). Brood size manipulation in the kestrel (*Falco tinnunculus*): effects on offspring and parent survival. *The Journal of Animal Ecology* **59**(1)**,** 269-285.

Dunn, E. (1979). Age of effective homeothermy in nestling Tree Swallows according to brood size. *Wilson Bulletin* **91**(3)**,** 455-457.

Erikstad, K. E., Bustnes, J. O., and Moum, T. (1993). Clutch size determination in precocial birds: a study of the common eider. *The Auk* **110,** 623-629.

Fisher, M. O., Nager, R. G., and Monaghan, P. (2006). Compensatory growth impairs adult cognitive performance. *Plos Biology* **4**(8)**,** 1462-1466.

Garnett, M. C. (1981). Body size, its heritability and influence on juvenile survival among great tits, *Parus major*. *Ibis* **123,** 31-41.

Houston, D. C., Jones, P. J., and Sibly, R. M. (1983). The effect of female body condition on egg laying in lesser black-backed gulls *Larus fuscus*. *Journal of Zoology* **200**(4)**,** 509-520.

Ilmonen, P., Hasselquist, D., and Langefors, A. (2003). Stress, immunocompetence and leukocyte profiles of pied flycatchers in relation to brood size manipulation. *Oecologia* **136,** 148-154.

Kitaysky, A. S., Kitaiskaia, E. V., Piatt, J. F., and Wingfield, J. C. (2003). Benefits and costs of increased levels of corticosterone in seabird chicks. *Hormones and Behavior* **43,** 140-149.

Kitaysky, A. S., Kitaiskaia, E. V., Piatt, J. F., and Wingfield, J. C. (2006). A mechanistic link between chick diet and decline in seabirds? *Proceedings of the Royal Society B-Biological Sciences* **273**(1585)**,** 445-450.

Kitaysky, A. S., Wingfield, J. C., and Piatt, J. F. (2001). Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake. *Behavioral Ecology* **12**(5)**,** 619-625.

Klomp, H. (1970). The determinatin of clutch size in birds: a review. *Ardea* **58,** 1-124.

Lack, D. (1947). The Significance of Clutch-Size. *Ibis* **89**(4)**,** 668-668.

Lacombe, D., Bird, D. M., and Hibbard, K. A. (1994). Influence of reduced food availability on growth of captive american kestrels. *Canadian Journal of Zoology* **72**(12)**,** 2084-2089.

Loonen, M. J. J. E., Bruinzeel, L. W., Black, J. M., and Drent, R. H. (1999). The benefit of large broods in barnacle geese: a study using natural and experimental manipulations. *Journal of Animal Ecology* **68**(4)**,** 753-768.

Love, O. P., Breuner, C. W., Vezina, F., and Williams, T. D. (2004). Mediation of corticosterone-induced reproductive conflict. *Hormones and Behavior* **46**(1)**,** 59-65.

Lynn, S. E., Breuner, C. W., and Wingfield, J. C. (2003). Short-term fasting affects locomotor activity, corticosterone, and corticosterone binding globulin in a migratory songbird. *Hormones and Behavior* **43**(1)**,** 150-157.

Martin, T. E. (1987). Food as a limit on breeding birds: a life-history perspective. *Annual Review of Ecology and Systematics* **18,** 453-487.

McCarty (2001). Variation in growth of nestling tree swallows across multiple temporal and spatial scales. *The Auk* **118**(1)**,** 176-190.

McCarty, J. P., and Winkler, D. W. (1999). Relative importance of environmental variables in determining the growth of nesting tree swallows *Tachycineta bicolor*. *Ibis* **141,** 286-296.

Metcalfe, N. B., and Monaghan, P. (2001). Compensation for a bad start: grow now, pay later? *Trends in Ecology & Evolution* **16**(5)**,** 254-260.

Monaghan, P., Bolton, M., and Houston, D. C. (1995). Egg production constraints and the evolution of avian clutch size. *Proceedings of the Royal Society* **259**(1355)**,** 189-191.

Monaghan, P., and Nager, R. G. (1997). Why don't birds lay more eggs? *Trends in Ecology & Evolution* **12**(7)**,** 270-274.

Murphy, M. T., Armbrecth, B., Vlamis, E., and Pierce, A. (2000). Is reproduction by tree swallows cost free? *The Auk* **117**(4)**,** 902-912.

Neuenschwander, S., Brinkhof, M. W. G., Kölliker, M., and Richner, H. (2003). Brood size, sibling competition, and the cost of begging in great tits (*Parus major*). *Behavioral Ecology* **14**(4)**,** 457-462.

Nowicki, S., Peters, S., and Podos, J. (1998). Song learning, early nutrition and sexual selection in songbirds. *American Zoologist* **38**(1)**,** 179-190.

Nur, N. (1984). The consequences of brood size for breeding blue tits II Nestling weight, offspring survival and optimal brood size. *Journal of Animal Ecology* **53,** 497-517.

Perrins, C. M. (1965). Population fluctuations and clutch-size in the great tit, *Parus major*. *Journal of Animal Ecology* **34,** 601-647.

Pravosudov, V. V. (2003). Long-term moderate elevation of corticosterone facilitates avain food-caching behaviour and enhances spatial memory. *Proceedings of the Royal Society B-Biological Sciences* **270,** 2599-2604.

Robertson, R. J., Stutchbury, B. J., and Cohen, R. R. (1992). Tree Swallow (Tachycineta bicolor), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology.

Robins, J. D. (1970). The relationship of food supply to the timing of breeding in aerial foragers. *Kansas Ornithological Society Bulletin* **21**(2)**,** 9-15.

Saino, N., de Ayala, R. M., Martinelli, R., and Boncoraglio, G. (2008). Male-biased brood sex ratio depresses average phenotypic quality of barn swallow nestlings under experimentally harsh conditions. *Oecologia* **156**(2)**,** 441-453.

Shutler, D., Clark, R. G., Fehr, C., and Diamond, A. W. (2006). Time and recruitment costs as currencies in manipulation studies on the costs of reproduction. *Ecology* **87**(11)**,** 2938-2946.

Siegel, H. S. (1980). Physiological Stress in Birds. *Bioscience* **30**(8)**,** 529-534.

Spear, L., and NUR, N. (1994). Brood Size, Hatching Order and Hatching Date - Effects on 4 Life-History Stages from Hatching to Recruitment in Western Gulls. *Journal of Animal Ecology* **63**(2)**,** 283-298.

Spencer, K. a., Buchanan, K. L., Goldsmith, A. R., and Catchpole, C. K. (2003). Song as an honest signal of developmental stress in the zebra finch (Taeniopygia guttata). *Hormones and Behavior* **44**(2)**,** 132-139.

Tinbergen, J. M., and Boerlijst, M. C. (1990). Nestling weight and survival in individual great tits (*Parus major*). *Journal of Animal Ecology* **59,** 1113-1127.

Wada, H., and Breuner, C. W. (2008). Transient elevation of corticosterone alters begging behavior and growth of white-crowned sparrow nestlings. *Journal of Experimental Biology* **211**(10)**,** 1696-1703.

Washburn, B. E., Morris, D. L., Joshua, J. M., Faaborg, J., and John, H. S. (2002). Using a commercially available radioimmunoassay to quantify corticosterone in avian plasma. *The Condor* **104**(3)**,** 558-563.

Weitzman, E. D., Fukushima, D., Nogeire, C., Roffwarg, H., Gallagher, T. F., and Hellman, L. (1971). Twenty-four hour pattern of the episodic secretion of cortisol in normal subjects. *Journal of Clinical Endocrinology* **33,** 14-22.

Wingfield, J. C., O'Reilly, K. M., and Astheimer, L. B. (1995). Modulation of the adrenocortical responses to acute stress in arctic birds: a possible ecological basis. *Integrative and Compartive Biology* **35**(3)**,** 285-294.

Wingfield, J. C., and Sapolsky, R. M. (2003). Reproduction and resistance to stress: when and how. *Journal of Neuroendocrinology* **15,** 711-724.

Winkler, D. (1993). Use and importance of feathers as nest lining in tree swallow (*Tachycineta bicolor*). *The Auk* **110**(1)**,** 29-36.

Table 2.1. Principal component loadings from principal component analyses (PCA) of hourly and daily weather variables. The weather from the range of dates and times (see text) were used to complete the PCA. The principal components were included in the analyses of nest visitation rates, chick mass, chick condition, chick growth rates and asymptotic mass and baseline corticosterone levels

Hourly Weather PCA		
Percent Explained: PC1	46.74%	
Percent Explained: PC2	23.44%	
Accumulated Percent Explained	70.18%	
PC Loadings	PC1	PC2
Air Temperature	0.54	0.26
Precipitation	-0.13	0.55
Relative Humidity	-0.55	0.17
Wind Speed	-0.004	0.78
Solar Radiation	0.63	0.04

Daily Weather PCA		
Percent Explained: PC1	39.3%	
Percent Explained: PC2	27.0%	
Accumulated Percent Explained	66.3%	
PC Loadings	PC1	PC2
Average High Temperature	-0.13	0.73
Average Low Temperature	0.28	0.66
Average Wind Speed	0.12	0.09
Daily Precipitation	0.47	-0.06
Average Relative Humidity	0.57	0.05

Table 2.2. Models used for analyzing the effects of brood size on nest visitation rates, chick mass, chick condition, growth rates and asymptotic mass calculated from a Gompertz model and chick plasma corticosterone levels in Tree Swallows. Analyses were performed on measurements collected on the indicated day post-hatch. Weather PC1 & 2 = principal component from weather date (see text).

	Nest Visitation Rates (visits/30min)	Mass (g)	Condition	Growth & Asymptotic Mass(g)	Corticosterone (ng/ml)
Days Post-hatch	7, 12 & 15[1]	3[1], 7[1], 12 & 15[1]	3[1], 7[1], 12 & 15[1]	3-15	12
Brood Size	✓	✓	✓	✓	✓
Year	✓	✓	✓	✓	✓
Julian Date	✓	✓	✓	✓	✓
Time of Day	✓	–	✓	–	✓
Weather PC1	✓	✓	✓	✓	✓
Weather PC2	✓	✓	✓	✓	✓

[1] Year is not included in the model for measurements made in only one year

Table 2.3. Average nest visitation rates of Tree Swallows near Ames, IA, in 2007 and 2008, during a 30-minute observation period across the brood sizes.

	Nest VR (visits/30 min)		Per Capita Chick Feeding Rates (visits/30 min)	
	Average	SD	Average	SD
Day 7	11.61	4.66	3.16	2.04
Day 12	13.51	6.95	3.44	1.67
Day 15	14.68	6.82	3.82	1.86

Table 2.4. Effect of brood size on nest visitation rates and estimated feeding rates per chick of Tree Swallow chicks near Ames, IA in 2007 and 2008 using a G^2 analysis. Visitation rates on day 15 were measured only in 2008. Effect direction is included for significant variables.

Day 7	Nest VR		Per Capita FR/Chick		
Sources of Variation	G^2_{df}	p-value	G^2_{df}	P-value	Effect Direction Nest VR/Per Capita FR
Model	13.33	0.21	34.86	<0.01	
Brood Size	$10.5_{5, 29}$	0.06	$24.27_{5, 29}$	<0.01	0.11/ -0.25
Year	$1.40_{1, 29}$	0.24	$1.40_{1, 29}$	0.24	
Julian Date	$1.87_{1, 29}$	0.17	$1.87_{1, 29}$	0.17	
Time of Day	$0.10_{1, 29}$	0.75	$0.10_{1, 29}$	0.75	
Weather PC1 (hourly)	$0.24_{1, 29}$	0.62	$0.24_{1, 29}$	0.62	
Weather PC2 (hourly)	$0.09_{1, 29}$	0.77	$0.09_{1, 29}$	0.77	
Day 12	Nest VR		Per Capita FR/Chick		
Sources of Variation	G^2_{df}	p-value	G^2_{df}	P-value	Effect Direction
Model	16.21	0.09	14.96	0.13	
Brood Size	$14.63_{5, 28}$	0.01	$8.10_{5, 38}$	0.15	0.21/ NS
Year	$0.42_{1, 28}$	0.52	$0.42_{1, 38}$	0.52	
Julian Date	$3.62_{1, 28}$	0.06	$3.6_{1, 38}$	0.06	
Time of Day	$0.501_{, 28}$	0.48	$0.50_{1, 38}$	0.48	
Weather PC1 (hourly)	$0.49_{1, 28}$	0.48	$0.49_{1, 38}$	0.48	
Weather PC2 (hourly)	$0.64_{1, 28}$	0.42	$0.64_{1, 38}$	0.42	
Day 15	Nest VR		Per Capita FR/Chick		
Sources of Variation	G^2_{df}	p-value	G^2_{df}	P-value	Effect Direction
Model	27.30	<0.01	27.6772	<0.01	
Brood Size	$22.12_{5, 9}$	<0.01	$13.46_{5, 9}$	0.02	0.18/ -0.18
Year	-	-	-	-	
Julian Date	$8.62_{1, 9}$	<0.01	$8.62_{1, 9}$	<0.01	0.05/ 0.05
Time of Day	$2.57_{1, 9}$	0.11	$2.5_{1, 9}$	0.11	
Weather PC1 (hourly)	$3.25_{1, 9}$	0.07	$3.25_{1, 9}$	0.07	
Weather PC2 (hourly)	$0.56_{1, 9}$	0.46	$0.56_{1, 9}$	0.46	

Table 2.5. Effect of brood size on mass of Tree Swallow chicks near Ames, IA in 2007 and 2008 using a G^2 analysis on days 3, 7, 12 and 15 post-hatch. Effect direction is included for significant variables.

Day 3			
Sources of Variation	G^2_{df}	p-value	Effect Direction
Model	13.07	0.11	
Brood Size	$9.10_{5, 31}$	0.11	
Year	$5.81_{1, 31}$	0.02	
Julian Date	$2.48_{1, 31}$	0.12	
Weather PC1 (daily)	$0.39_{1, 31}$	0.53	
Weather PC2 (daily)	$4.81_{1, 31}$	0.03	
Day 7			
Sources of Variation	G^2_{df}	p-value	Effect Direction
Model	19.83	0.02	
Brood Size	$17.1_{5, 36}$	<0.01	-0.82
Year	$0.66_{1, 36}$	0.42	
Julian Date	$0.12_{1, 36}$	0.73	
Weather PC1 (daily)	$0.29_{1, 36}$	0.59	
Weather PC2 (daily)	$0.02_{1, 36}$	0.90	
Day 12			
Sources of Variation	G^2_{df}	p-value	Effect Direction
Model	23.80	<0.01	
Brood Size	$15.8_{5, 37}$	<0.01	-0.39
Year	$0.41_{1, 37}$	0.52	
Julian Date	$0.12_{1, 37}$	0.73	
Weather PC1 (daily)	$7.91_{1, 37}$	<0.01	1.38
Weather PC2 (daily)	$1.37_{1, 37}$	0.24	
Day 15			
Sources of Variation	G^2_{df}	p-value	Effect Direction
Model	26.62	<0.01	
Brood Size	$20.2_{5, 38}$	<0.01	-0.44
Year	$<0.01_{1, 38}$	0.99	
Julian Date	$8.08_{1, 38}$	<0.01	-0.10
Weather PC1 (daily)	$2.46_{1, 38}$	0.12	
Weather PC2 (daily)	$0.48_{1, 38}$	0.49	

Table 2.6. Average mass (g) of Tree Swallow chicks across all brood sizes.

	Mass (g)	
	Average	SD
Day 3	5.64	0.90
Day 7	16.19	1.98
Day 12	22.23	1.52
Day 15	21.43	1.69

Table 2.7. Effect of brood size on condition of Tree Swallow chicks near Ames, IA in 2007 and 2008 using a G^2 analysis on days 3, 7, 12 and 15 post-hatch. Condition on days 3, 7 and 15 post-hatch were from 2008 only, but day 12 post-hatch data are from 2007 and 2008. Effect direction is included for significant

Day 3			
Sources of Variation	G^2_{df}	p-value	Effect Direction
Model	7.8	0.45	
Brood Size	$1.4_{4, 12}$	0.85	
Year	-	-	
Julian Date	$1.7_{1, 12}$	0.19	
Time of Day	$3.2_{1, 12}$	0.07	
Weather PC1	$0.5_{1, 12}$	0.50	
Weather PC2	$2.7_{1, 12}$	0.10	
Day 7			
Sources of Variation	G^2_{df}	p-value	Effect Direction
Model	20.4	0.01	
Brood Size	$12.3_{4, 11}$	0.02	-0.85
Year	-	-	
Julian Date	$3.60_{1, 11}$	0.06	
Time of Day	$<0.01_{1, 11}$	0.95	
Weather PC1	$2.30_{1, 11}$	0.13	
Weather PC2	$2.40_{1, 11}$	0.12	
Day 12			
Sources of Variation	G^2_{df}	p-value	Effect Direction
Model	33.5	<0.02	
Brood Size	$15.8_{5, 28}$	0.01	-0.03
Year	$10.7_{1, 28}$	<0.01	1.17
Julian Date	$<0.01_{1, 28}$	0.94	
Time of Day	$0.50_{1, 28}$	0.46	
Weather PC1	$7.30_{1, 28}$	0.01	0.99
Weather PC2	$15.0_{1, 28}$	<0.01	-2.86
Day 15			
Sources of Variation	G^2_{df}	p-value	Effect Direction
Model	18.6	0.02	
Brood Size	$13.7_{5, 14}$	0.02	-0.46
Year	-	-	
Julian Date	$4.00_{1, 14}$	0.05	-0.28
Time of Day	-	-	
Weather PC1	$2.20_{1, 14}$	0.14	
Weather PC2	$0.50_{1, 14}$	0.47	

Table 2.8. Effect of brood size on growth rates and predicted asymptotic mass of Tree Swallow chicks near Ames, IA in 2007 and 2008. Growth rates and asymptotic mass were calculated using a Gompertz logistic growth curved fitted to chick mass measurements from each nest on days 3, 7, 12 and 15 post-hatch. Effect direction is included for significant variables.

	Growth Rates		
Sources of Variation	G^2_{df}	p-value	Effect Direction
Model	5.47	0.79	
Brood Size	$2.40_{5, 27}$	0.79	
Year	$0.04_{1, 27}$	0.84	
Julian Date	$0.33_{1, 27}$	0.57	
Weather PC1 (0-15)	$2.49_{1, 27}$	0.11	
Weather PC2 (0-15)	$0.26_{1, 27}$	0.61	
	Asymptotic Mass		
Sources of Variation	G^2_{df}	p-value	Effect Direction
Model	17.6	0.04	
Brood Size	$12.8_{5, 27}$	0.03	-0.64
Year	$0.15_{1, 27}$	0.70	
Julian Date	$2.4_{1, 27}$	0.12	
Weather PC1 (0-15)	$4.99_{1, 27}$	0.03	1.80
Weather PC2 (0-15)	$0.19_{1, 27}$	0.66	
	Predicted Age to reach Asymptotic Mass		
Sources of Variation	G^2_{df}	p-value	Effect Direction
Model	7.5	0.58	
Brood Size	$2.64_{5,26}$	0.76	
Year	$0.01_{1,26}$	0.93	
Julian Date	$1.25_{1,26}$	0.26	
Weather PC1 (0-15)	$2.83_{1,26}$	0.09	
Weather PC2 (0-15)	$0.25_{1,26}$	0.62	

Table 2.9. Effect of brood size on plasma baseline CORT levels (ng/ml) of Tree Swallow chicks near Ames, IA in 2007 and 2008 using a G^2 analysis. CORT levels are from blood samples collected on day 12 post-hatch. Effect direction is included for significant variables.

	Baseline CORT		
Sources of Variation	G^2_{df}	p-value	Effect Direction
Model	24.61	<0.01	
Brood Size	$4.82_{5, 27}$	0.44	
Year	$14.3_{1, 27}$	<0.01	0.63
Julian Date	$0.44_{1, 27}$	0.51	
Time of Day	$0.15_{1, 27}$	0.70	
Weather PC1 (previous hour)	$0.19_{1, 27}$	0.66	
Weather PC2 (previous hour)	$0.11_{1, 27}$	0.74	

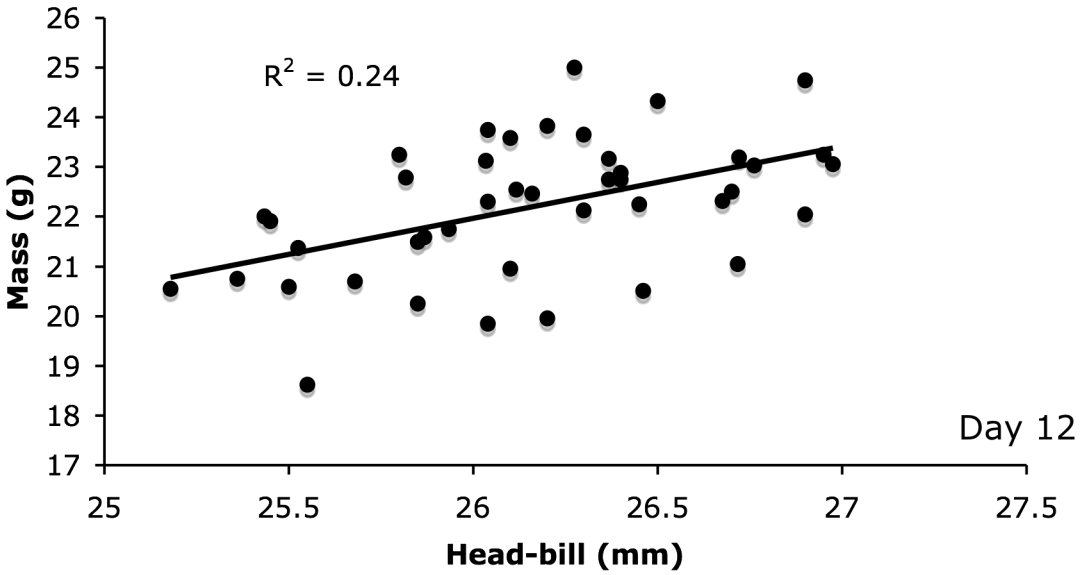


Figure 2.1. Head-bill and mass measurements and regression line plotted to calculate the residuals that were used for body condition in Tree Swallows in central Iowa, 2007-2008. Residuals were calculated separately for each day of measurements (i.e., 3, 7, 12 and 15 post-hatch).

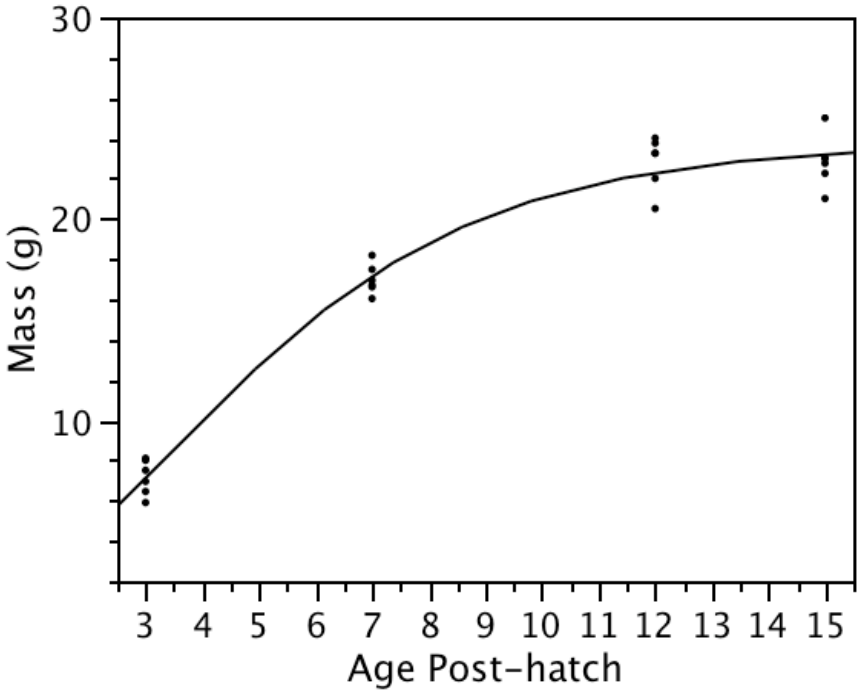


Figure 2.2. A representative Gompertz logistic growth curve fitted to mass measurements of a 5-chick Tree Swallow brood from one nest on days 3, 7, 12 and 15 post-hatch, 2007-2008.

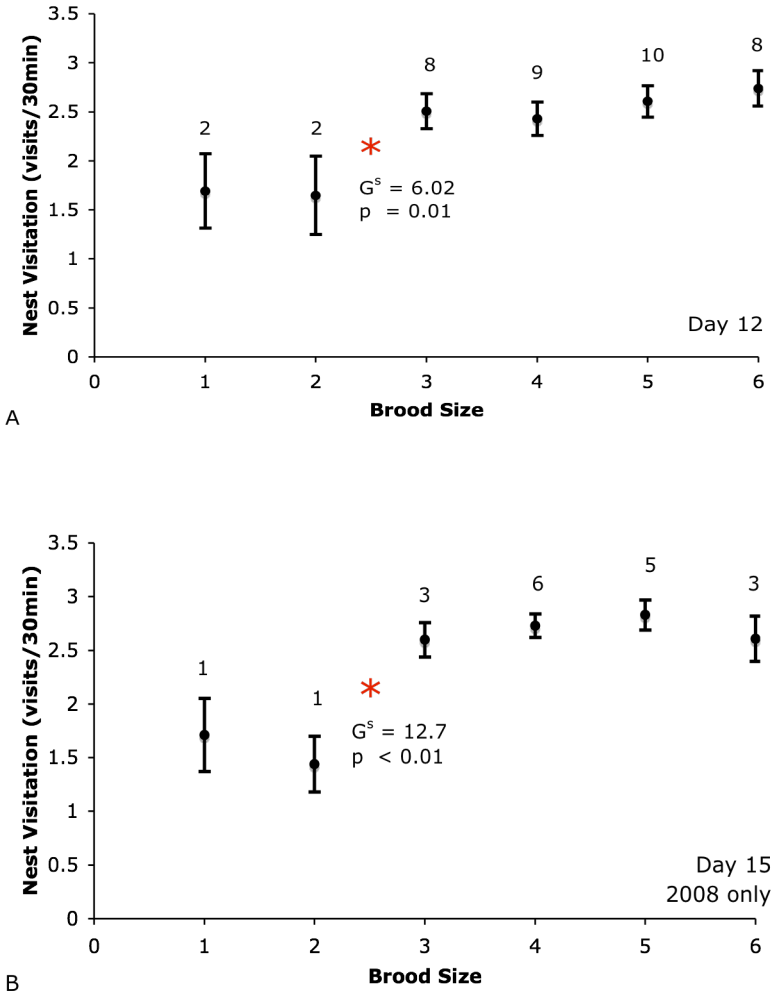


Figure 2.3. Least square means of nest visitation rates of Tree Swallow adults in 2007 and 2008 as a function of brood size on days 12 (A) and 15 (B) post-hatch. Data are ln-transformed. Sample sizes for broods of the indicated size are shown above the data points and vertical lines represent the standard error bars. The * denotes a significant difference between the respective brood sizes with statistics shown.

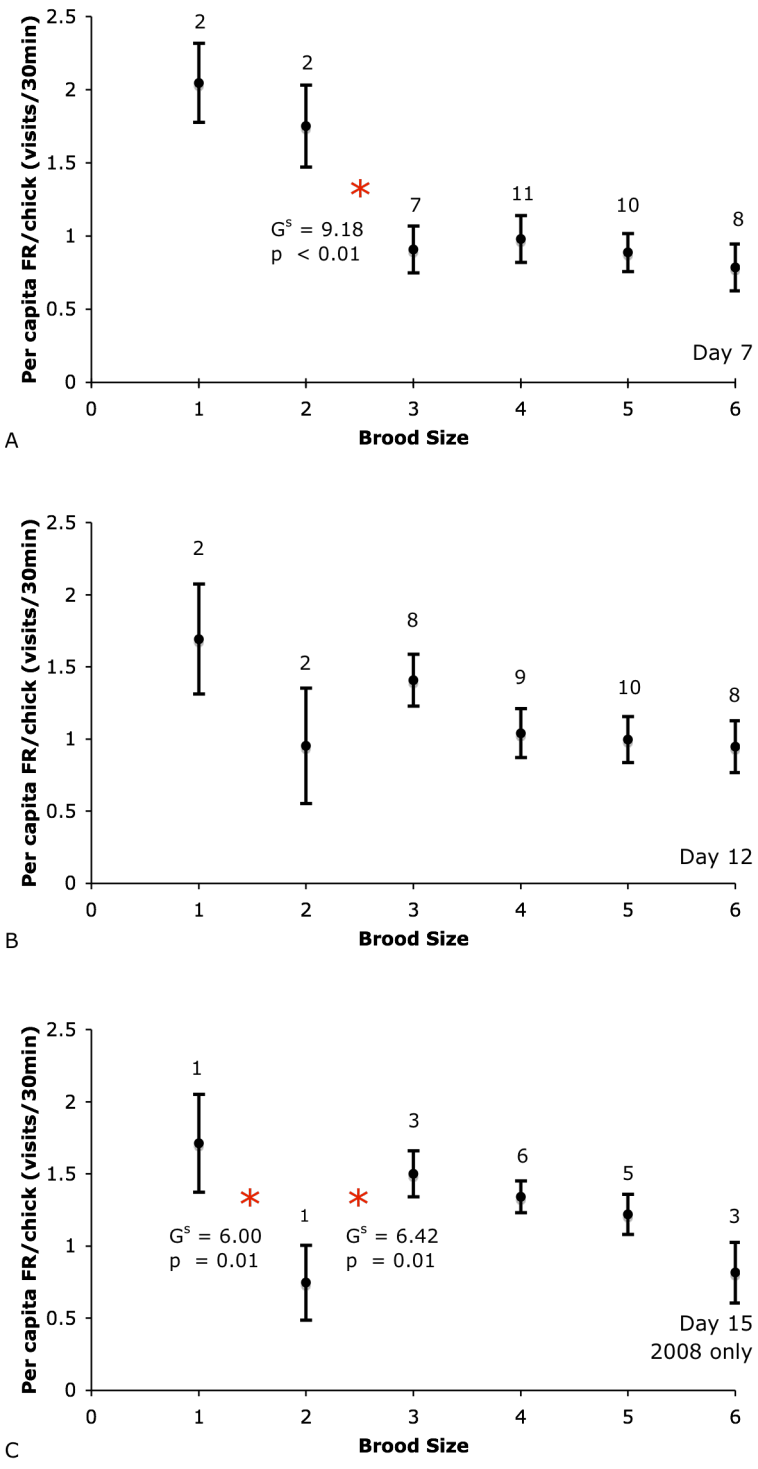


Figure 2.4. Least square means of per capita Tree Swallow chick feeding rates in 2007 and 2008 as a function of brood size on days 7(A), 12(B) and 15(C) post-hatch. Data as in Fig. 2.

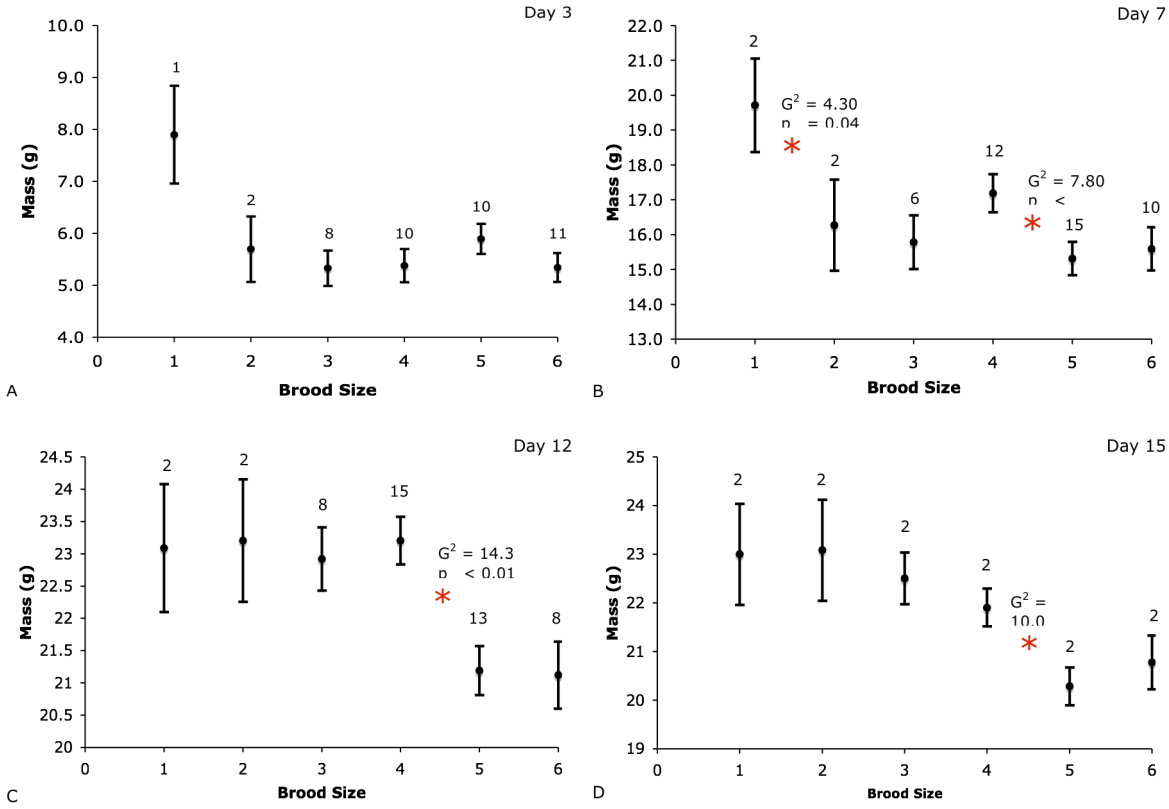


Figure 2.5. Least square means of Tree Swallow chick mass in 2007 and 2008 as a function of brood size on days 3(A), 7(B), 12(C) and 15(D) post-hatch. Data as in Fig. 2.

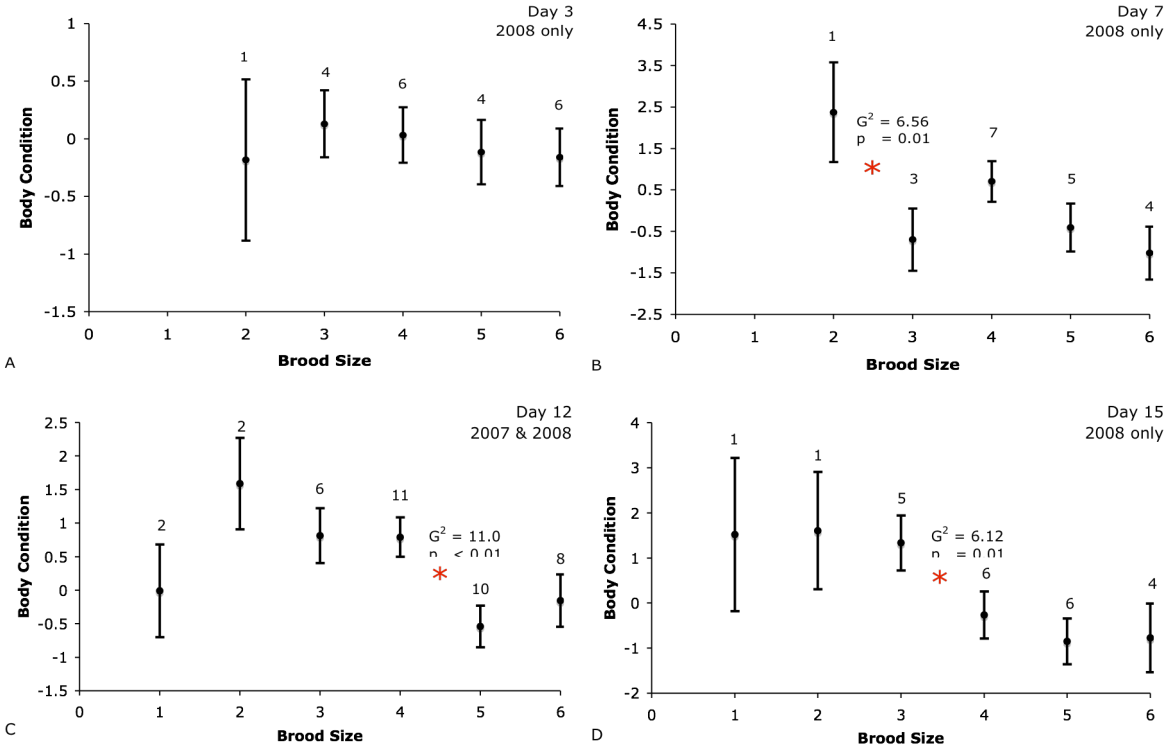
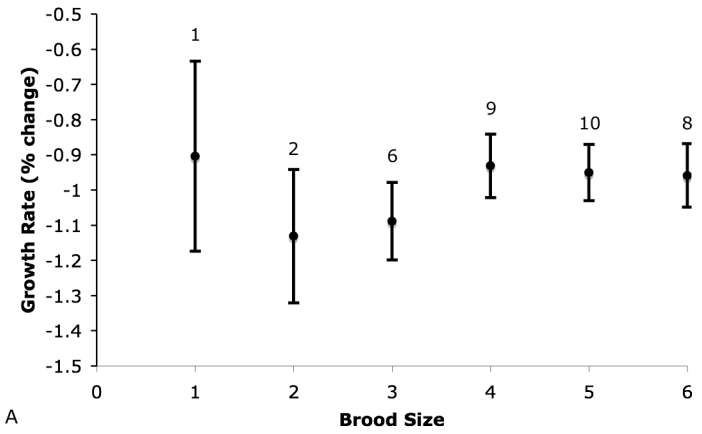
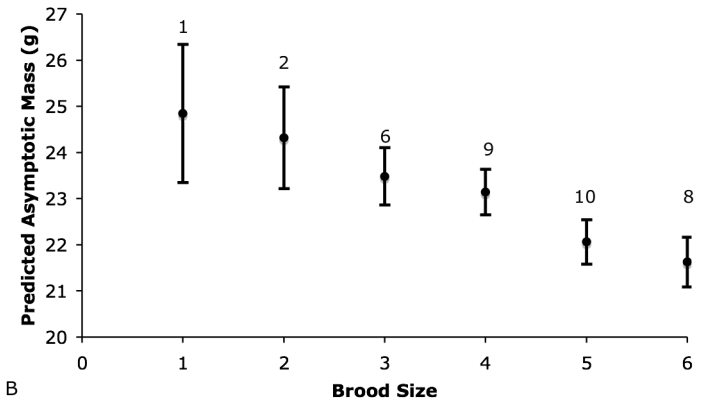


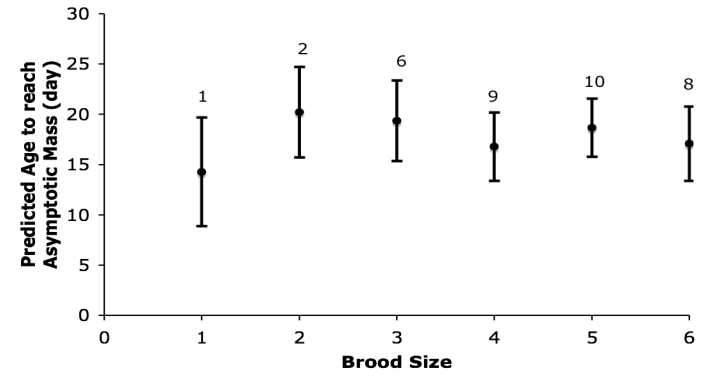
Figure 2.6. Least square means of Tree Swallow chick body condition as a function of brood size on days 3(A), 7(B), 12(C) and 15(D) post-hatch. Body condition was calculated using the residuals of mass versus head-bill for all chicks separately for each day. There was no significant effect of brood size on chick body condition on day 3 post-hatch (A). Data are as in Fig. 2.



A



B



C

Figure 2.7. Least square means of the ln-transformed growth rates (A), predicted asymptotic mass (B) and predicted age to reach asymptotic mass (C) of Tree Swallow chicks as a function of brood size. These were both calculated using a Gompertz logistic growth curve fitted to chick mass measurements from each nest on days 3, 7, 12 and 15 post-hatch. Data are as in Fig. 2. There was no significant affect of brood size on the chick growth rates (A).

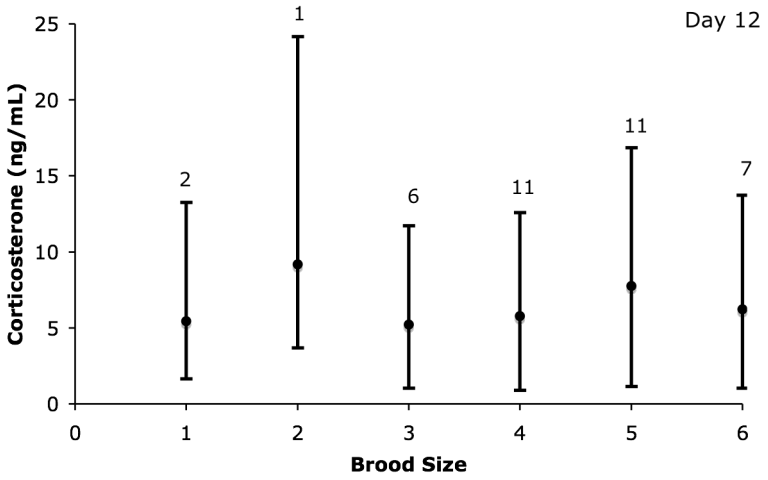


Figure 2.8. Least square means of baseline corticosterone levels in Tree Swallow chicks as a function of brood size on day 12 post-hatch. For analyses the CORT data were ln-transformed and the resulting least square means were de-transformed for the figure. Data are as in Fig. 2.

CHAPTER 3
ONTOGENY OF BASELINE AND STRESS LEVELS OF CORTICOSTERONE IN NESTLING TREE SWALLOWS (*Tachycineta bicolor*)

INTRODUCTION

Animals experience numerous unpredictable perturbations or challenging events throughout their lives, such as inclement weather, food shortage, social conflict, predator/prey interactions, etc. The stress response is an important adaptation that induces behavioral and physiological modifications enabling survival during such events. The stress response is a coping mechanism for short-term perturbations, mediated by the hypothalamic-pituitary-adrenal (HPA) axis, which regulates the release of glucocorticoids from the adrenal glands (Siegel, 1980). The increase in glucocorticoids has functions in several tissues, which are critical for immediate survival. Glucocorticoids increase cardiovascular tone, mobilize stored energy, enhance memory formation and suppress sexual behaviors (Sapolsky, 2000). Corticosterone (CORT) is the main glucocorticoid in birds and most mammals and has been the hormone of study in stress physiology research. The stress response is adaptive for short-terms stressors, but despite the clear benefits of the acute response to stress, there are costs to maintaining elevated CORT levels for prolonged periods of time. These costs might be especially high in developing young.

A variety of costs have been reported from studies investigating the effects of prolonged elevations of CORT. Individuals with chronically elevated CORT levels are more susceptible to disease due to suppression of the immune system (reviewed in McEwen, et al., 1997; Sapolsky, 2000; Loiseau, et al., 2008). Individuals also experience high energetic demands due to the mobilization of fuels (Siegel, 1980; Sapolsky, 2000), and there appear to be effects on developmental trajectories and overall reduced fitness (Kitaysky, et al., 2003; Fisher, et al., 2006; Kitaysky, et al., 2006; Hull, et al., 2007; Spencer and Verhulst, 2007; Loiseau et al., 2008; Wada and Breuner, 2008). For example, Black-legged Kittiwake (*Rissa tridactyla*) chicks with experimentally elevated CORT levels during the nestling period had reduced cognitive abilities compared to chicks with blank implants (Kitaysky et al., 2003). In

addition, chicks with CORT implants increased their food intake, but their masses were not different from control chicks, likely due to the high energy demands from the elevated CORT levels. In another study male Zebra Finches (*Taenopigia guttata*) that experienced prolonged elevations in CORT levels during the nestling period suffered impaired song learning (Spencer, et al., 2003). These males sang significantly shorter songs than control males in adulthood, suggesting that the experimental males were of lower quality. Therefore, although elevations in CORT levels can benefit young in foraging and survival during the early stages of independence (Heath, 1997), there are potentially severe, long-term costs to prolonged elevations in CORT in developing young. This suggests there should be strict regulation of the HPA axis in young.

Studies have demonstrated a hypo-responsive period to stress in rat pups (*Rattus norvegicus*) (reviewed in Sapolsky and Meaney, 1986) and in rainbow trout (*Oncorhynchus mykiss*) (Barry, et al., 1995). During early stages of development young respond to a stressor with lower levels of glucocorticoids than do young later in development or as adults. It is thought that this hypo-responsive period is due to repression of the HPA axis and is adaptive to avoid the potential costs of elevated CORT levels during critical developmental stages (Barry et al., 1995; Sapolsky, 2000). There have been few studies on birds investigating the development of the stress response, but those published have found chicks early in the nestling period have lower stress response CORT levels than older chicks (Schwabl, 1999; Sims and Holberton, 2000; Sockman and Schwabl, 2001; Love, et al., 2003; Fridinger, et al., 2007; Wada, et al., 2007; Quillfeldt, et al., 2009). It is unclear whether this is due to an undeveloped or repression of the HPA axis.

The objective of this study was to investigate the ontogeny of baseline and stress response levels of CORT in nestling Tree Swallows (*Tachycineta bicolor*). This species produces altricial young so I have two predictions based on what has been found thus far in other altricial species (Sims and Holberton, 2000; Wada et al., 2007): 1) there will be an increase in baseline CORT levels prior to fledging; 2) chicks early in the nestling period will exhibit lower stress levels of CORT compared to older chicks.

METHODS

The Tree Swallow (*Tachycineta bicolor*) is a migratory passerine that breeds in the northern United States and Canada, typically from May to July (Robertson, et al., 1992). They are secondary-cavity nesters that nest readily in provided nest boxes. Furthermore, Tree Swallows are aerial insectivores that prefer to nest in open areas near water. The central Iowa population has a clutch size range of 1-7 eggs with an average of 5.3 ± 1.0 eggs per clutch. Brood sizes have been documented to range between 1 to 7 chicks in our population with an average of 3.9 ± 2.1 chicks per brood based on chicks that fledge (Parsons, *pers. obs.),* Raising and fledging more than one brood in a season is rare, however re-nesting does occur if nest failure occurs early enough in the season. Only female Tree Swallows incubate and generally begin incubation on the day the penultimate egg is laid. Incubation lasts, on average, 14 days. Most clutches hatch over a 1-2 day period. Chicks fledge from the nest by 18-20 days post-hatch.

Study Site

The study was conducted during 2007 and 2008 using a wild population of Tree Swallows at three locations near Ames, Iowa with 205 nest boxes distributed between the sites. Each site contains access to prairie and water. The sites are located at Ada Hayden Park (42^{o}32'N, 93^{o}37'W), ISU Horticulture Farm (42^{o}6'N, 93^{o}35'W) and Hinds Research Farm (42^{o}3'N, 93^{o}37'W). Nests were monitored every three days beginning in early May to record nest building activity. Once egg-laying began I checked the nests every 2-3 days to record the date of egg-laying and clutch size, and then every day around the time of hatching (approximately 14 days after the start of incubation) in order to record the hatch date.

Blood Sampling

I measured baseline CORT by obtaining a blood sample within 3 minutes of first disturbing the chicks and I measured the stress-induced CORT levels by subjecting birds to a uniform stressor (20 minutes of handling) and then obtaining a second blood sample. Chicks were bled on one of three sampling days (day 7, 12 or 15 post-hatch) with 1-2 chicks per nest bled on each sample day, so that each chick was bled only once. On each sample day all the

chicks were handled in the same manner, so even though chicks were not bled on a given day they were handled for the same amount of time. Sampled chicks had a blood sample taken from the brachial vein within 3 minutes of removal from the nest and again 20 minutes post-removal from the nests. I collected ~25 ul of blood at each time in heparinized microhemotocrit tubes, which were kept on ice until I returned to the lab within 7 hours. In the lab, I spun the blood in a hematocrit centrifuge and transferred the plasma to 0.5ml centrifuge tubes, which were stored in at -80^{o}C until analyzed. During the 20-minute stress period chicks on days 7 and 12 post-hatch were kept in a divided cardboard box that kept the chicks separated. Chicks were kept in a cloth bag on day 15 post-hatch for the 20-minute handling period. Prior to banding, I identified individual chicks by unique nail polish colors on all claws of one foot.

I sampled from 10 nests each in 2007 and 2008 for a total of 20 nests.

Weather Parameters

Weather data for Ames, IA, located at 42° 1'N, 93° 47'W, were obtained from the Iowa Ag Climate Network (http://mesonet.agron.iastate.edu/agclimate/index.phtml). I acquired hourly and daily weather data from the summers of 2007 and 2008, which included temperature (average high and low temperature for the daily weather data), precipitation, wind speed and relative humidity.

Corticosterone Assay

I assayed plasma concentrations of CORT using a double antibody I^{125} radioimmunoassay (RIA) kit from MP Biomedicals (Catalog # 07-120103, MP Biomedical, Orangeburg, NY). Samples were run in triplicate each year. This kit has been validated for measuring plasma CORT concentrations in birds (Washburn, et al., 2002). I followed the protocol of the kit with the exception of quartering the volume of all the reagents, diluting the 25 ng/ml standard to 1:2 and 1:4 with steroid diluent to produce a 12.5 ng/ml standard and a 6.25 ng/ml standard. I also used a 1:40 dilution of plasma, using the provided steroid diluent in the assay. This dilution resulted in percent binding that fell consistently within the usable range provided by the standard curve. A pool of Tree Swallow plasma was used to test for binding

parallelism with the standard curve. The intra-assay variation was 7% and the inter-assay variation was 15%. The inter-assay variation was calculated using the average coefficient of variation of the control samples provided in the kit, while the intra-assay variation was calculated using the average coefficient of variation of each set of triplicate. The control samples were run with every set of samples through the assay. Plasma from each year was run in separate assays at the end of their respective field seasons.

Statistical Analyses

All analyses were performed using JMP statistical software (SAS Institute 2007). I used the mean of the measurements for the chicks sampled on each day within each nest for the analyses. I used the generalized linear model platform that uses a likelihood ratio chi-square (G^2) for all analyses using an α-level = 0.05. Model designs were based on including independent variables of interest (i.e., baseline or stress response CORT) and other factors that are known to have effects on CORT levels: age of chicks, Julian data, time of day, weather and year (Dawson, et al., 2005; Dawson, 2008). For the purpose of this study I were primarily interested in the effects of age of the chicks on CORT levels and not effects of various weather variables; therefore I used principal component analyses (PCA) to combine our weather data. Hourly weather data were used to calculate the weather PCA: I used hourly weather data collected during the range of dates over which I collected samples, 14 June – 25 July, and times, 0630 – 1400 hours. The principal components from the hour prior to the collection of the blood sample were used in the analysis of CORT. The principal component loadings of the weather PCA can be found in Table 3.1.

RESULTS

Baseline CORT levels were significantly positively correlated with age post-hatch and year (Table 3.2, Fig 3.1). The average baseline CORT levels showed a 33% increase from day 12 to day 15 (Table 3.3, Fig 3.1).

The stress response CORT levels increased with age, year and Julian date (Table 3.2, Fig 3.2). Chicks on day 7 post-hatch had significantly lower stress CORT levels than chicks on

days 12 and 15 post-hatch (Table 3.2, Fig 3.2), but these levels were higher than their baseline CORT levels (Fig 3.3), indicating they do exhibit a stress response.

DISCUSSION

Tree Swallow nestlings showed an increase in baseline CORT levels with respect to age. Chicks 15 days post-hatch had significantly higher CORT levels than chicks 7 and 12 days post-hatch. Increasing baseline CORT late in the nestling period has been seen in other bird species: American Kestrels (Heath, 1997; Sockman and Schwabl, 2001; Love et al., 2003), Eastern and Western Screech Owls (*Otus asio* and *O. kennicottii*, respectively) (Belthoff and Alfred M. Dufty, 1998),White-crowned Sparrows (Wada et al., 2007). Although Love et al. (2003) found a relatively linear increase in baseline CORT with age in American Kestrels, others have found lower baseline CORT levels in the early stages of the nestling period and higher CORT levels near the end of the nestling period.

The increase in baseline CORT by day 15 post-hatch may function to prepare the chicks for fledging. An increase in CORT levels prior to fledging is thought to help newly fledged young survive (Belthoff and Alfred M. Dufty, 1998). Altricial nestlings spend the first part of their lives confined to a nest and must transition quickly to an active stage of flying and foraging. Increased CORT may facilitate this transition in young birds by inducing locomotor activity and mobilizing stored energy when chicks fledge (Belthoff and Alfred M. Dufty, 1998).

CORT has been studied in relation to juvenile dispersal in Eastern and Western Screech Owls during which juveniles move from their natal range to new territories (Belthoff and Alfred M. Dufty, 1998). CORT is thought to play an important role in initiating the necessary physiological and behavioral modifications necessary to survive the demands of establishing and defending a new territory (Belthoff and Alfred M. Dufty, 1998). This is similar to the physiological and behavioral modifications necessary prior to fledging.

Tree Swallow nestlings of all ages sampled exhibited a stress response to the 20-minute

restraint stressor with elevations in CORT above baseline levels. Tree Swallow chicks 7 days post-hatch, however, responded to the 20-minute restraint with lower CORT levels than chicks 12 and 15 days post-hatch. This may indicate that at some point along the HPA axis there are components that are not fully developed and are not responsive to a stressor and thus, there is a period during the early nestling period when Tree Swallow chicks reduce their responsiveness to stress.

It takes time and significant resources for a growing organism to develop. A review by Jenkins and Porter (2004) showed that the chicken embryo goes through several stages of development in the HPA axis, but the HPA axis is fully developed prior to hatching. Chickens are precocial birds with chicks that are feathered and mobile at hatch. At hatch altricial chicks, such as Tree Swallows, are featherless with their eyes closed and are essentially helpless. Altricial birds may not have an HPA axis as fully developed as in precocial birds. Altricial species are less developed at hatch, which may result in a time early in the nestling period where chicks do not respond to a stressor or at least not as strongly as older chicks. Wada et al. (2007) found a similar pattern in Nutall's White-crowed Sparrows (*Zonotrichia leucophrys nuttalli*) which have chicks early in the nestling period that show lower stress levels of CORT to a stressor than older chicks. They also found that chicks 1 to 3 days post-hatch responded less to an ACTH injection than older chicks, indicating that the adrenal glands may have a role in a lower stress response CORT levels in younger chicks.

Another possibility is that this period of lower stress response could be due to repression of the HPA axis during the period shortly after hatching resulting in a period when the HPA axis would exhibit a decreased response to a stressor. Studies in rats have shown that before birth fetal rats have CORT levels that increase up to parturition (Sapolsky and Meaney, 1986). After birth rat pups go through a hypo responsive period to induced stressors (ie. handling stress and ACTH injections). It is thought that this is an adaptive modification to the HPA axis to avoid the potentially negative costs from elevated CORT levels during critical developmental times (Sapolsky and Meaney, 1986). Studies in altricial birds that investigated the ontogeny of the stress response argue that nestlings go through the same modification in

the HPA axis and that the hypo responsive period is adaptive (Wada et al., 2007; Quillfeldt et al., 2009) as found in the rats, but it is not known when the HPA axis is fully developed in altricial species.

Our results are similar to what others have found in patterns of stress response CORT (Schwabl, 1999; Sockman and Schwabl, 2001; Love et al., 2003; Wada et al., 2007). Literature is lacking in altricial species, however, making it impossible to infer whether the low stress response relative to older chicks is due to an underdeveloped HPA axis or to a repressed HPA axis. It is necessary to know when the HPA axis is fully functioning and responsive to stimuli. This will aid in understanding if low stress response CORT levels in young chicks are due to the immaturity or repression of the HPA axis.

BIBLIOGRAPHY

Barry, T. P., Malison, J. A., Held, J. A., and Parrish, J. J. (1995). Ontogeny of the cortisol stress response in larval rainbow trout. *General and Comparative Endocrinology* **97,** 57-65.

Belthoff, J. R., and Alfred M. Dufty, J. (1998). Corticosterone, body condition and locomotor activity: a model for dispersal in screech-owls. *Animal Behavior* **55,** 405-415.

Dawson, R. D. (2008). Timing of breeding and environmental factors as determinants of reproductive performance of tree swallows. *Canadian Journal of Zoology* **86,** 843-850.

Dawson, R. D., Lawrie, C. C., and O'Brien, E. L. (2005). The importance of microclimate variation in determining size, growth and survival of avian offspring: experimental evidence from a cavity nesting passerine. *Oecologia* **144,** 499-507.

Fisher, M. O., Nager, R. G., and Monaghan, P. (2006). Compensatory growth impairs adult cognitive performance. *Plos Biology* **4**(8)**,** 1462-1466.

Fridinger, R. W., O'Reilly, K. M., Kildaw, S. D., and Buck, C. L. (2007). Removal of a nest-mate elicits an age-dependent increase in plasma corticosterone of nestlng black-legged kittiwakes. *Journal of Field Ornithology* **78**(1)**,** 93-99.

Heath, J. (1997). Corticosterone levels during nest departure of juvenile american kestrels. *The Condor* **99**(3)**,** 806-811.

Hull, K. L., Cockrem, J. F., Bridges, J. P., Candy, E. J., and Davidson, C. M. (2007). Effects of corticosterone treatment on growth, development, and the corticosterone response to handling in young Japanese quail. *Comparative Biochemistry and Physiology a-Molecular & Integrative Physiology* **148**(3)**,** 531-543.

Jenkins, S. A., and Porter, T. E. (2004). Ontogeny of the hypothalamic-pituitary-adrenocortical axis in the chicken embryo: a review. *Domestic Animal Endocrinology* **26,** 267-275.

Kitaysky, A. S., Kitaiskaia, E. V., Piatt, J. F., and Wingfield, J. C. (2003). Benefits and costs of increased levels of corticosterone in seabird chicks. *Hormones and Behavior* **43,** 140-149.

Kitaysky, A. S., Kitaiskaia, E. V., Piatt, J. F., and Wingfield, J. C. (2006). A mechanistic link between chick diet and decline in seabirds? *Proceedings of the Royal Society B-Biological Sciences* **273**(1585)**,** 445-450.

Loiseau, C., Sorci, G., Dano, S., and Chastel, O. (2008). Effects of experimental increase of corticosterone levels on begging behavior, immunity and parental provisioning rate in house sparrows. *General and Comparative Endocrinology* **155**(1)**,** 101-108.

Love, O. P., Bird, D. M., and Shutt, L. J. (2003). Plasma corticosterone in american kestrel siblings: effects of age, hatching order, and hatching asynchrony. *Hormones and Behavior* **43,** 480-488.

McEwen, B. S., Biron, C. A., Brunson, K. W., Bulloch, K., Chambers, W. H., Dhabhar, F. S., Goldfarb, R. H., Kitson, R. P., Miller, A. H., Spencer, R. L., and Weiss, J. M. (1997). The role of adrenocorticoids as modulators of immune function in health and disease: Neural, endocrine and immune interactions. *Brain Research Reviews* **23**(1-2)**,** 79-133.

Quillfeldt, P., Poisbleau, M., Chastel, O., and Masello, J. F. (2009). Acute stress hyporesponsive period in nestling thin-billed prions *Pacyptila belcheri*. *Journal of Compartive Physiology: A* **195,** 91-98.

Robertson, R. J., Stutchbury, B. J., and Cohen, R. R. (1992). Tree Swallow (Tachycineta bicolor), The Birds of North America Online (A. Poole, Ed.). Ithaca: Cornell Lab of Ornithology.

Sapolsky, R. M. (2000). Stress hormones: Good and bad. *Neurobiology of Disease* **7**(5)**,** 540-542.

Sapolsky, R. M., and Meaney, M. J. (1986). Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. *Brain Research Reviews* **11,** 65-76.

Schwabl, H. (1999). Developmental changes and among-sibling variation of corticosterone levels in an altricial avian species. *General and Comparative Endocrinology* **116,** 403-408.

Siegel, H. S. (1980). Physiological Stress in Birds. *Bioscience* **30**(8)**,** 529-534.

Sims, C. G., and Holberton, R. L. (2000). Development of the corticosterone stress response in young northern mockingbirds (*Mimus polyglottos*). *General and Comparative Endocrinology* **119,** 193-201.

Sockman, K. W., and Schwabl, H. (2001). Plasma corticosterone in nestling american kesterls: effects of age, handling stress, yolk androgens, and body condition. *General and Comparative Endocrinology* **122,** 205-212.

Spencer, K. a., Buchanan, K. L., Goldsmith, A. R., and Catchpole, C. K. (2003). Song as an honest signal of developmental stress in the zebra finch (Taeniopygia guttata). *Hormones and Behavior* **44**(2)**,** 132-139.

Spencer, K. a., and Verhulst, S. (2007). Delayed behavioral effects of postnatal exposure to corticosterone in the zebra finch (Taeniopygia guttata). *Hormones and Behavior* **51**(2)**,** 273-280.

Wada, H., and Breuner, C. W. (2008). Transient elevation of corticosterone alters begging behavior and growth of white-crowned sparrow nestlings. *Journal of Experimental Biology* **211**(10)**,** 1696-1703.

Wada, H., Hahn, T. P., and Breuner, C. W. (2007). Development of stress reactivity in white-crowned sparrow nestlings: total corticosterone response increases with age, while free corticosterone response remains low. *General and Comparative Endocrinology* **150,** 405-413.

Washburn, B. E., Morris, D. L., Joshua, J. M., Faaborg, J., and John, H. S. (2002). Using a commercially available radioimmunoassay to quantify corticosterone in avian plasma. *The Condor* **104**(3)**,** 558-563.

Table 3.1. Principal component loadings from principal component analyses (PCA) of hourly weather variables. The weather from the range of dates and times, see text, were used to complete the PCA. The principal components were included in the analyses of corticosterone levels in Tree Swallows.

Hourly Weather PCA		
Percent Explained: PC1	46.74%	
Percent Explained: PC2	23.44%	
Accumulated Percent Explained	70.18%	
PC Loadings	PC1	PC2
Air Temperature	0.54	0.26
Precipitation	-0.13	0.55
Relative Humidity	-0.55	0.17
Wind Speed	-0.004	0.78
Solar Radiation	0.63	0.04

Table 3.2. Effect of age on baseline and stress response CORT levels in Tree Swallow chicks near Ames, IA in 2007 and 2008 using a G^2 analysis.

	Baseline CORT (ng/ml)		Stress Response CORT (ng/ml)	
Sources of Variation	G^2_{df}	p-value	G^2_{df}	p-value
Model	27.57	<0.01	14.21	0.03
Age Post-hatch	$8.80_{1, 25}$	<0.01	$4.04_{5, 24}$	0.04
Year	$15.7_{1, 25}$	<0.01	$7.92_{1, 24}$	<0.01
Julian Date	$1.48_{1, 25}$	0.22	$1.82_{1, 24}$	0.18
Time of Day	$0.29_{1, 25}$	0.59	$0.01_{1, 24}$	0.91
Weather PC1 (previous hour)	$0.16_{1, 25}$	0.69	$0.34_{1, 24}$	0.56
Weather PC2 (previous hour)	$0.13_{1, 25}$	0.71	$2.51_{1, 24}$	0.11

Table 3.3. Average baseline and stress response levels of CORT in nestling Tree Swallows in 2007 and 2008 of different ages.

	Baseline CORT (ng/ml)		Stress Response CORT (ng/ml)	
	Average	SD	Average	SD
Day 7	5.92	3.49	43.02	17.28
Day 12	6.57	4.22	63.55	23.19
Day 15	11.50	6.05	65.77	21.47

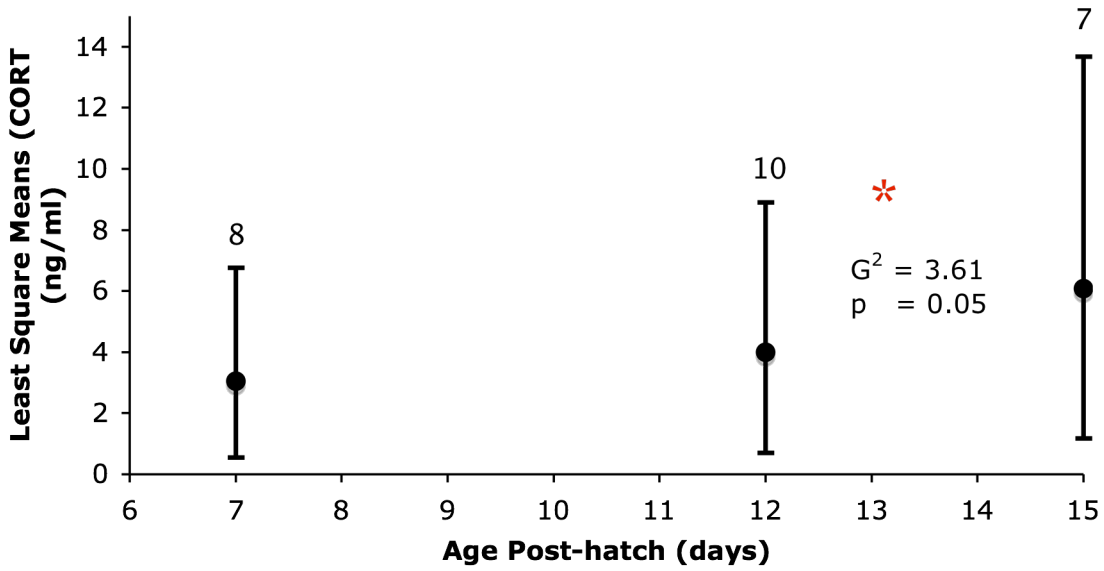


Figure 3.1. Least square means of baseline CORT ng/ml as a function of age post-hatch in Tree Swallow chicks in 2007 and 2008. Sample sizes for the indicated ages are above the data points and vertical lines represent the standard error bars. The * denotes a significant difference between the respective ages (statistics shown). Data were ln-transformed before analyses, the resulting least square means were de-transformed for

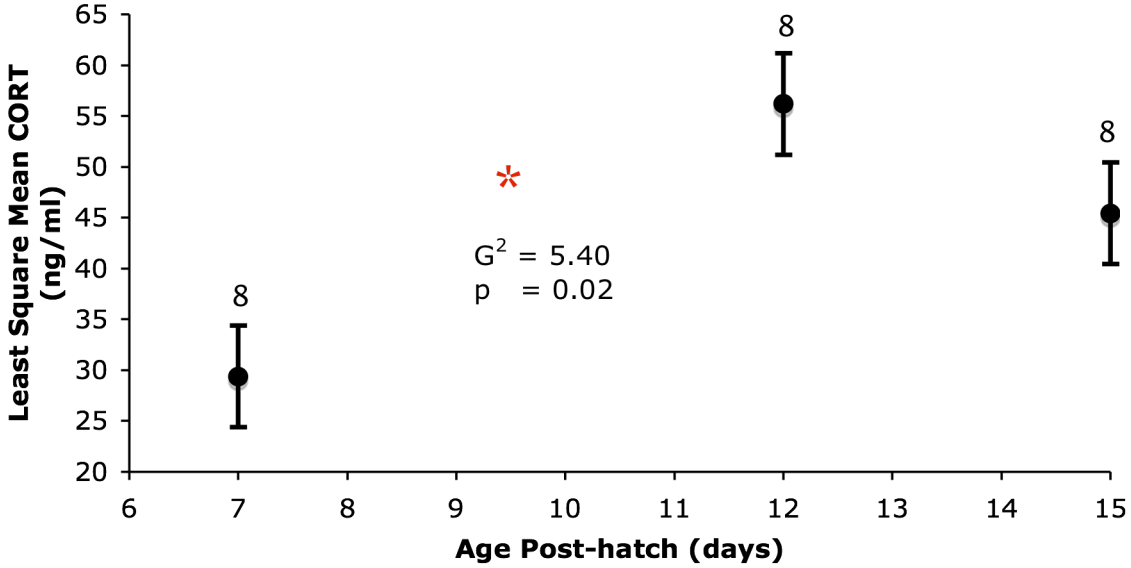


Figure 3.2. Least square means of stress response levels of CORT ng/ml as a function of age post-hatch in Tree Swallow chicks in 2007 and 2008. Sample sizes for the indicated ages are above the data points and vertical lines represent the standard error bars. The * denotes a significant difference between the respective ages (statistics shown).

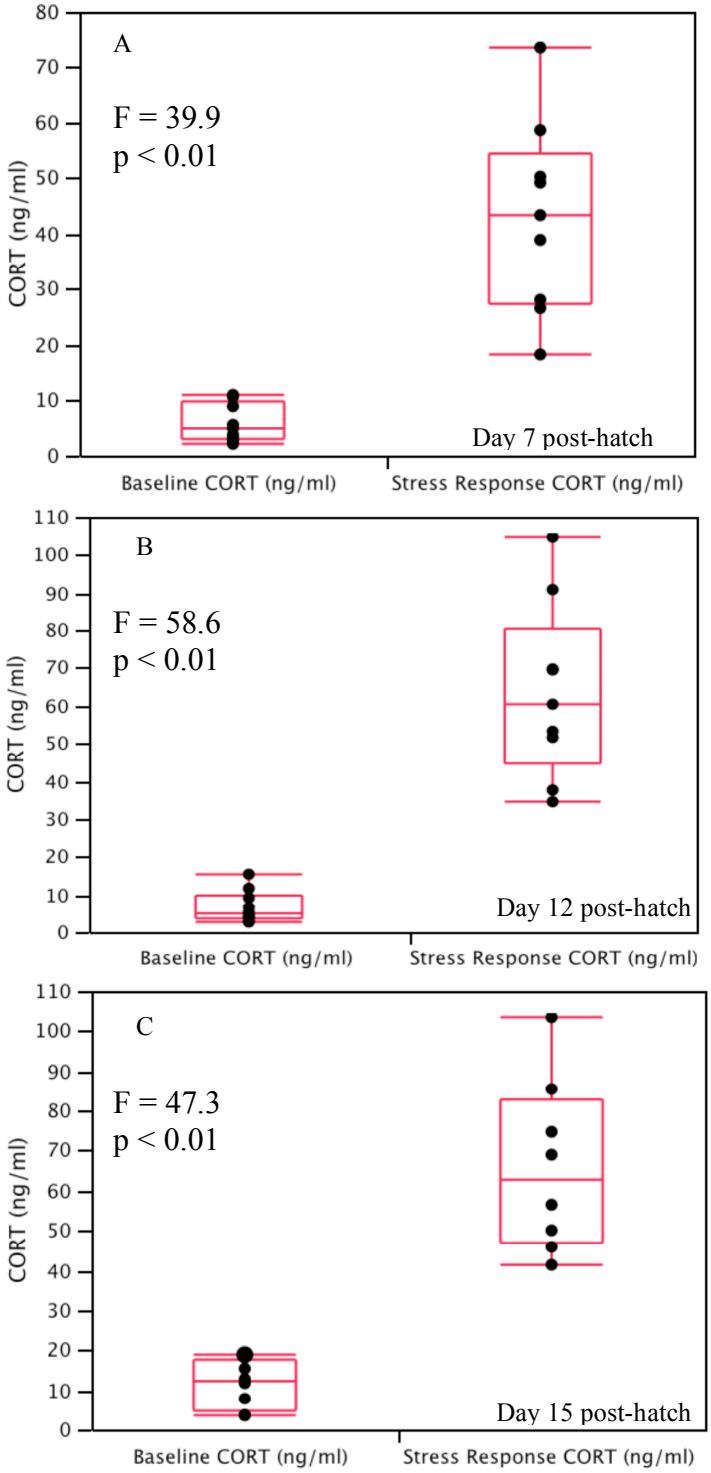


Figure 3.3. ANOVA of baseline and 20-minute stress response levels of CORT in Tree Swallow chicks on days 7 (a), 12 (b) and 15 (c) post-hatch in 2007 and 2008.